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RESEARCH ARTICLE

Diagnosis of the Causes of Vaginal Candidiasis in Pregnant Women

Noor Alhuda Mohsen Zahi[1]

[1]College Of Medicine, Wasit University, Kut, Iraq.

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Corresponding author:

Noor Alhuda Mohsen Zahi College of Medicine, Wasit University, Kut, Iraq.

ABSTRACT

Objective: The aim of the study was to diagnose the types that cause vaginal candidiasis among pregnant women in Karbala.

Methods: Vaginal swabs were collected from pregnant women with symptoms of vaginal infections returning to the Obstetrics and Gynecology Hospital in Karbala. The samples were diagnosed by two methods of direct examination, by culture on plates and by supporting the diagnosis using Vitek device. The samples were cultured on a Sabouraud dextrose agar (SDA) medium. They incubated the dishes at a temperature of 37ºC for 48 to 72 hours to allow the development of colonies. After the emergence of growth on the plates, the samples are examined using the Vitek device, which is characterized by the ability to determine the types of yeasts and the identification of the type of organism. After then, the antifungal activity of free kenaconzole and nanohybrid kenacozole was tested against *Candida prapsilosis*.

Results: The results showed that there are nine samples that appeared yeast after culture them in SDA. The results of diagnoses by Vitek showed that four species were *C. parapsilosis*, three were *C. albicans* and two werer *C. famata*. The statistical analysis results showed that the diameter of inhibition increases significantly ($p \leq 0.05$) in the nano hybrid ketoconazole and free kenaconazloe if a concentration was increased.

Conclusions: According to the current study, there are no common species of *Candida* sp. in pregnant women. The success of loading kenconazole on zinc oxide as a carrier of the antibiotic. The activity of the kenacozole and zinc oxide nano-hybrid compound gave a higher inhibition activity than free ketoconazole.

INTRODUCTION

Among all the childhood motivations most comprehensive and strongest. Motherhood is a beautiful and joyful experience for a woman. The feeling that it shows in the value that appears in the beginning.[1] The common diseases afflict a pregnant woman, her midriff and sweat, presented more fear and anxiety for her fetus and is seen in most cases of pregnant women than pregnant women.

Usually women have a vaginal discharge, which appears in many cases that are not satisfactory. Then, the abnormal vaginal secretions result from vulvovaginitis, including bacterial vaginosis [BV], candidiasis or trichomoniasis.[2]

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Vulvovaginal candidiasis appears to occur more often when estrogen levels are increased, such as the use of oral contraceptives (especially high estrogen doses), pregnancy and estrogen. Vaginal secretions during pregnancy decrease from an alkaline pH to 4 or 5 (an acidic pH). This is caused by the action of *Lactobacillus acidophilus.*

Vulvovaginal Candidiasis (VVC), an opportunistic mucosal mycosis caused by *Candida albicans,* is one of the most common causes of vaginitis.[3,4] Its incidence has increased markedly during the last three decades.[4] Approximately 75% of all pregnant women experience at least one episode of VVC during their lifetime and 50% of them suffer recurrent events.[5,6] The incidence of VVC is doubled in the third trimester of pregnancy and multigravida suffer significantly more than primigravida.[6,7]

Moreover, a significant proportion of women with chronic or recurrent Candidiasis first present with this infection while pregnant. They appear for the first time during pregnancy.[7,8]

Vaginal candidiasis can cause miscarriage, candidiasis, amnioticitis, candidiasis and face childbirth. Premature neonates are at high risk of fungal infection due to an immature ovarian system during childbirth, which leads to a high spread of infection from the vagina of the infected mother to the newborn, which leads to a rise in congenital *Candida* infection. Infants with thrush, it can lead to candidiasis of the nipple when breastfeeding. Nonetheless, Natalia and Maaleh research is an outcome of the research in Prenatal Therapy. Medicines that contain preferred medicines.[9]

Vulvovaginal candidiasis can be diagnosed by visualization of yeast hyphae on potassium hydroxide preparation a woman with typical symptoms. It can also be diagnosed using antigen or DNA probe testing, with sensitivities of 77 to 97% and specificities of 77 to 99%, compared with culture as the diagnostic standard. Acidic vaginal pt. recurrent (four or more episodes in one year) or severe infections, or infections that occur in a patient who is Women with vulvovaginal candidiasis have a normal complicated vulvovaginal candidiasis is defined as immunocompromised such as someone with AIDS or poorly controlled diabetes mellitus. Culture is particularly important for diagnosing and treating complicated vulvovaginal candidiasis because patients are more likely to have nonalbicans infection.[10]

The aim of the study to diagnose the types that cause vaginal candidiasis in pregnant women in Karbala governorate.

MATERIALS AND METHODS

The media and the equipment required in our study are summarized in the bellow Table 1.

Sample Collection

Vaginal swabs were obtained from pregnant women who frequented the Obstetrics and Gynecology Hospital in Karbala and its private outpatient clinics. The ages of the women with vaginal candidiasis ranged between 18 to 34 years.

Samples were collected during the period from February 1 to February 10, 2021, and information about each patient›s age, place of residence and number of previous births were recorded.

Table 1: Materials and equipment

No	*Materials and equipment*	*Company and country of manufacture*
1.	Sabouraud dextrose Agar	Hi Media Laboratories Pvt. Ltd.
2.	Nutrient agar & broth agar	Lab M Limited topely House, 52 wash Lane Bury, Lancashire BL9 6As, United Kindom
3.	Petri dishes	China
4.	Filter papers	
5.	Cork borer	China
6.	Sensitive balanced	Germany
7.	benzen flame	China
8.	Magnetic mixer	China
9.	Vitek2_compressed	Boimerieux
10.	Incubation	Germany
11.	Autoclave	Korea
12.	Microscope	Germany
13.	Micropipette	Germany /Human
14.	Tong	Korea
15.	Glass flask	China
16.	Tube	Iraq
17.	Cotton	China
18.	ethanol	Korea
19.	Amoxycillin	China

Preparation of media

Nutrient Agar: Suspend 28g of nutrient agar powder in 1L of distilled water. Mix and dissolve them completely. Sterilize by autoclaving at 121°C for 15 minutes. Pour the liquid into the petri dish and wait for the medium to solidify (leave for thirty minutes). Be sure that you are preparing the agar in the clean environment to prevent any contamination. Notice Once the agar solidifies, the agar is ready to use

Nutrient Broth: weigh 25 grams of powder, disperse in 1L of deionized water Allow to soak for 10 minutes swirl to mix then dispense into tubes or bottles and sterilise for 15 minutes at 121ºC.

Sabouraud Dextrose Agar (SDA): Suspend 65 gm of the medium in 1000 mL of distilled water. Heat to boiling to dissolve the medium completely, sterilize by autoclaving at 15 Ibs. We put an antibiotic, amoxicillin, to kill bacteria and prevent them from multiplying and spreading. Autoclave at 121°C for 15 minutes. Cool to 45 to 50°C and pour into petri dishes.

Culture

Candida species grow easily on Sabouraud dextrose agar medium, the common medium used to isolate *Candida* species, which allows the growth of *Candida* and inhibits the growth of some bacteria due to the low pH.

This method involves streak-growing the infected samples on a dish containing sabouraud dextrose agar medium near the Bunsen flame to ensure that no contamination occurs during cultivation. The dishes are incubated at 37 °C for 48-72 hours to allow the development of fungal colonies.[11,12]

Macroscopic and Microscopic Features

The colonies of *Candida* spp. are cream colored to yellowish, grow rapidly and mature in 3 days. The texture of the colony may be pasty, smooth, glistening or dry, wrinkled and dull, depending on the species.[13]

The microscopic features of *Candida* spp. also show species-related variations. All species produce blastoconidia singly or in small clusters. Blastoconidia may be round or elongate. Most species produce pseudohyphae which may be long, branched or curved. True hyphae and chlamydospores are produced by strains of some *Candida* spp.

Preparation of Nanohybrid Ketoconazole

The nanohybrid antibiotic was prepared using the process defined by Kolekar *et al*., (2011).[13]

- **Ketoconazole Solution:** This solution was prepared by dissolving 0.5 gm of the ketoconazole in an amount of 50% ethanol, and after completing the dissolution process, the volume was completed to 50 mL using ethanol as well.
- **Zinc Oxide Solution:** This solution was prepared by dissolving 1 gm of the zinc oxide in an amount of 50% ethanol, and after completing the dissolution process, the volume was completed to 50 mL using ethanol as well.
- **Nanohybrid Ketoconazole:** Fifty mL of ketoconazole solution was added drop by drop to 50 mL Zinc oxid solution, and the mixture was magnetically stirred at room temperature for 2 hours then placed in a shaking incubator at 37°C for 18 hours followed by 24 hours incubation in a static incubator at 40°C. The mixture was centrifuged at 5000 rpm for 20 minutes, washed several times with deionized water and finally dried. KEN-ZnO and KEN-Free were assigned to the prepared nanohybrid antibiotic and free ketoconazole, respectively.

Preparation of drug concentration

Using a sensitive balance, dissolve about 0.2 g of ketoconozole in 100 mL of distilled water and place in a clean, sterile tube. We prepared five concentrations in different tubes for each of nano and free at the ratio (0, 25, 50, 75, 100) respectively, and we dilute the tubes to get the required concentration (Figure 1).

- Pre-prepared nutrient agar plates with *Candida* ssp. in tubes from nutrient broth pierced with a cork borer for antibiotic application

Note: Each plate and tube must be numbered in order for the publishing process to take place in its correct form, as we took by micro-pipette an amount of the antibiotic found in the tube numbered on it no. 100 and put it in the middle of the plate numbered with the same number and published by a special publishing tool, and the dishes were observed at a temperature of 37°C for 24 hour, and the same way works for the rest of the prohibited dishes (Figure 2).

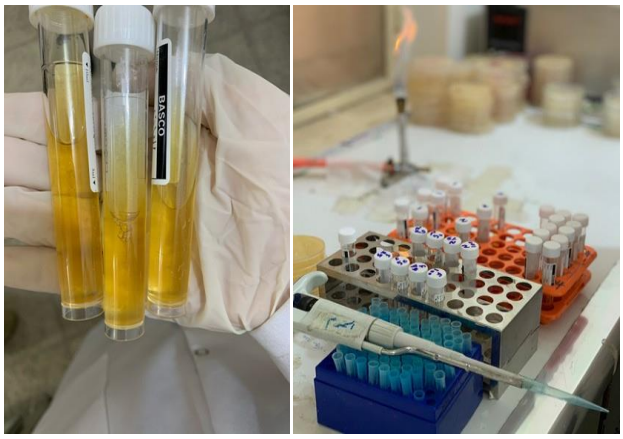

Figure 1: The preparation of broth media and drug concentrations

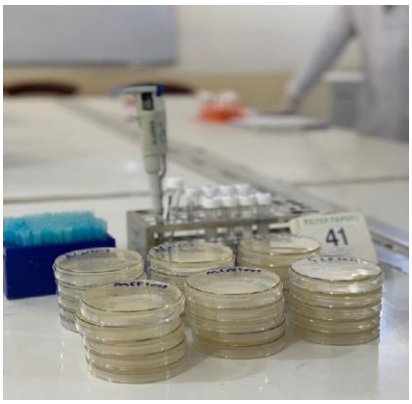

Figure 2: Petri dishes with media

Measurement of antifungal activity of Nanohybrid Ketoconazole:

KEN-ZnO, KEN-Free, were tested against *C. parapsilosis* using the agar well diffusion method defined by Egorove (1985),[14] with antibiotic concentrations ranging from 25 to 100 mg/mL:

Activation of *Candida*: The yeast *Candida* sp. grown on SDA medium was activated by transferring a whole loop from a single isolated colony to 5 mL of Nutrient broth medium and incubated at 35-37ºC for 18 hours thereafter. It was diluted with distilled water of the diagnostic kit to obtain a turbidity equivalent to 3 MacFarland.

Antimicrobial Activity assay: The inhibitory activity of the nanohybrid ketoconazole was tested against *C. parapsilosis* yeast according to diffusion agar method (Egorove, 1985) according to the following:

- The inoculum was diluted to obtain the equivalent of 0.5 MacFarland by comparing it to MacFarland.
- The inoculum was spread on a plate containing 20 mL of SDA well by swab (swab) and then left for an hour.
- Using a cork porrer with a diameter of 8 mm to make wells on the surface of the dishes, as the distance was equal between one hole and another.
- Graduated concentrations of nanohybrid ketoconazole as well as free ketoconazole ranging from (25–100) mg/mL were prepared by dissolving them in the distal water.

Table 2: Diagnostic results of biochemical tests for *Candida* isolates by Vitek.

Tests	*C. albicans (1)*	*C. albicans (2)*	*C. albicans (3)*	*C. famata (1)*	*C. famata (2)*	*C. parapsilosis (1)*	*C. parapsilosis (2)*	*C. parapsilosis (3)*	*C. parapsilosis (4)*
LysA	_	_	_	_	_	_	_	_	_
TyrA	+	_	_	+	_	_	_	+	_
dGLUa	+	+	+	+	+	+	+	+	+
dRAFa	_	_	_	_	+	_	_	_	_
IRHAa	_	_	_	+	_	_	_	_	_
dTURa	+	+	+	+	+	+	+	+	+
IGLTa	+	+	+	+	+	+	+	+	+
IPROa	+	+	+	+	+	+	+	+	+
IMLTa	_	_	_	+	_	_	_	_	_
BNAG	_	_	_	_	_	_	_	_	_
LACa	_	_	_	+	+	_	_	_	_
NAGA1	+	+	+	_	_	_	_	_	_
XLTa	+	+	+	_	+	_	_	_	_
dTREa	+	+	+	+	+	+	+	+	+
dXYLa	+	+	+	+	+	+	+	+	+
2KGa	+	+	+	+	+	+	+	+	+
LeuA	+	+	+	+	+	+	+	+	+
ARBa	_	_	_	_	+	_	_	_	_
MAdGa	+	+	+	+	+	+	+	+	_
dMNEa	+	+	+	+	+	+	+	+	+
dSORa	+	+	+	+	+	+	+	+	+
NO3a	_	_	_	_	_	_	_	_	_
LATa	+	+	+	_	+	_	_	_	_
NAGa	+	+	+	+	+	+	+	+	_
ARG	+	+	+	+	+	+	+	+	+
AMYa	_	_	_	_	+	_	_	_	+
dCELa	_	_	_	_	_	_	_	_	+
dMELa	_	_	_	_	+	_	_	_	+
SACa	+	+	+	+	+	+	+	+	_
IARAa	_	_	_	+	+	+	+	+	+
ACEa	+	+	+	+	+	+	+	+	_
dGNTa	+	+	+	+	+	+	+	+	+
ERYa	_	_	_	_	_	_	_	_	_
dGALa	+	+	+	+	+	+	+	+	_
GGT	_	_	_	_	_	_	_	_	+
dMLZa	_	_	_	+	_	+	+	+	+
URE	_	_	_	_	_	_	_	_	_
dGATa	+	+	+	_	+	+	_	+	_
CITa	+	+	+	+	+	+	+	+	+
GLYLa	_	_	_	+	+	+	+	+	+
GENa	_	_	_	_	+	_	_	_	_
dMALa	+	+	+	+	+	+	+	+	+
ISBEa	_	_	_	+	_	+	+	+	+
AGLU	+	+	+	+	+	+	+	+	+
ESC	_	_	_	_	_	_	_	_	_
GRTas	+	+	+	+	+	+	+	+	+

- One hundred microliters of each concentration of the nanohybrid ketoconazole under study were placed in each hole, and incubated for one hour in the refrigerator, and then incubated in the incubator at 37 °C for 24 hours.
- One hundred microliters of each concentration of the free ketoconazole were placed in each hole, and incubated for one hour in the refrigerator, and then incubated in the incubator at 37 °C for 24 hours.
- The diameters of yeast growth inhibition (mm) were measured using a ruler after the incubation period was completed.

Statistical Analysis

The statistical analysis included determination of the significant differences between the factors studied in this study by use of the Chi – square test and ANOVA one way test at $\alpha = 0.05$ (the probability level) (Dequ and Tessema, 2005).[15]

RESULTS AND DISCUSSION

Isolation and diagnostic

12 samples were collected by vaginal swabs from patients attending the obstetrics and gynecology hospital and private laboratories in Karbala governorate.

All the samples we obtained were subjected to a series of laboratory treatments. The sample was initially cultured on Sabouraud dextrose agar using clean and sterile soaps and near the Bunsen flame to avoid contamination. The dishes were kept at a temperature of 37ºC for 72 hours. During microscopic examination, dense growth of the required fungus was found.

Culture Results

The colonies growing on the SDA showed in the form of white to cream-colored, smooth, and circular colonies [Figure 3] In this regard, Ellis and his group (2007) indicate that *Candida* spp. colonies possess such phenotypic characteristics when planted on the mentioned medium.[16,17] This result agrees with the memorandum of Sing and his group (2013) with the emergence of cream-colored, shiny, smooth, and circular colonies to provide suitable cultivation conditions.[18] The colonies were diagnosed as *Candida* sp.

Microscopic features

The isolated species gave positive results for interaction with Gram stain, where the cells appeared oval to spherical or oval to elongated or cylindrical in the yeast shape of the fungus, and this result was identical with Boon and his group (2013) as shown in Figure 4 and that the appearance of *Candida* cells pigmented in blue color as a result of the retention of the peptidoglycan layer present in the cell wall of this dye.[19]

Diagnosis of *Candida* sp.

The results of *Candida* diagnosis by vitek through biochemical tests show that four isolated was *C. parapsilosis*, two isolated as C. famata, and three isolated as *C. albicans* , as shown in Tables 2 and 3. According to Table 3 nine isolates were

Figure 3: Growth of C. albicans on sabroide dextrose agar medium at a temperature 37ºC for one week

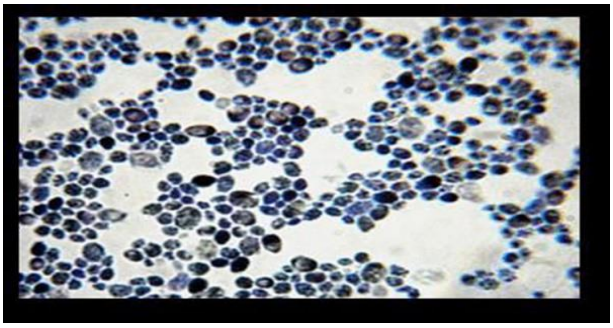

Figure 4: *Candida* sp. at 40 X magnification

diagnosed with an isolation rate of (0.7165) from the total samples (12), where 3 species of *Candida* spp. were isolated and diagnosed. In the current study by using a vitek device and based on the biochemical characteristics, it was isolated from different clinical samples that included the vagina and urine in different percentages [33.33, 22.22, 44.45%], respectively, as shown in Tables 2 and 3.

The results also showed that the percentage of *C. parapsilosis* isolate represented the largest percentage of the isolated species from the different clinical samples mentioned above, Table 3. This would contradict with (Al_obadi, 2012) if five species belonging to the genus *Candida* were isolated from the oral cavity, vagina and urethra. *C. albicans* came in preface.[20]

Antimicrobial activity

The results of the statistical analysis in Table 4 showed that there was a high significant difference ($p \leq 0.05$) in the diameters of the inhibition zone of the free ketoconazole against *C. parapsilosis* at the concentrations 25, 50, 75 and 100 µg/mL compared with the control. The diameters of the inhibition zone were 19, 33.5, 37 and 56 mm; respectively.

Table 3: The types of *Candida* that isolated from patients

The species	*Number*	*Percentage %*	*p-value*
C. albicans	3	33.33	
C. famata	2	22.22	0.7165
C. parapsilosis	4	44.45	
Total	9	100	

Table 4: The inhibitory efficacy of Ketoconazole against *C. parapsilosis* isolated from patients

Type of antifungal	*Concentration (µg/mL)*					*P value*	*LSD*
	O (Control)	*25*	*50*	*75*	*100*		
Ketoconazole (Free)	0 ± 0.00	19.5 ± 3.53	33.5 ± 2.12	37 ± 1.41	56 ± 1.41	0.0000**	4.42
Ketoconazole (Nano)	0 ± 0.00	14 ± 1.41	18 ± 2.82	24 ± 1.41	43.5 ± 9.19	0.0000**	10.27
P value	1.000	0.0053*	0.0001**	0.0001**	0.0081*		

The numbers refer to mean ± Standard Deviation
* refers to significance differences ($p \leq 0.05$)
** refers to high significance differences ($p \leq 0.001$)

The results of the statistical analysis in Table 4 showed that there was a high significant difference ($p \leq 0.05$) in the diameters of inhibition zone of the nanohybrid ketoconazole against *C. parapsilosis* at the concentrations 25, 50, 75 and 100 µg/mL compared with the control, also these concentrations did show high significant difference ($p \leq 0.05$) in the diameters of inhibition when compared with each other. The diameters of inhibition zone were 14, 18, 24 and 43.5 mm, respectively.

If comparing the free ketoconazole and nanohybrid ketoconazole, the results of the statistical analysis showed that the diameter of inhibition increases significantly ($p \leq 0.05$) in the nanohybrid ketoconazole at all concentrations of compared with the free ketoconazole, diameters of inhibition zone.

These results show improvement the efficiency of ketoconazole by using the nanotechnology method.

CONCLUSION

According to the results obtained from our current study, we can draw several conclusions, the most important of which are: The more species that isolated were *C. parabicellosis*. The success of loading ketoconazole on zinc oxide as a carrier of the antibiotic. The activity of the nano-hybrid compound of ketoconazole and zinc oxide gave a higher inhibition activity compared to free ketoconazole.

RECOMMENDATIONS

Recommendations can be summed up in the following studies:

1. Expanded the spectrum of the study to include the different area.
2. Preparation other antifungal agents by nano methods by using another carrier and tested it.
3. Study of the release of ketoconazole and the periods of release in different liquids in its acidity.

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