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A STUDY ON THE PATTERN OF *CANDIDA* SPECIES SENSITIVITY TO ANTIFUNGAL DRUGS AND ZNO NANOPARTICLE

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ABSTRACT

In recent years, systemic fungal infections caused by *Candida* species have been regarded as one of the most important factors of death. The outbreak of fungal infections has led to increasing use of antifungal drugs and considerable increase resistance of *Candida* species against them. Therefore, the present study was carried out to investigate the drug sensitivity of *Candida* agents isolated from the vaginal samples of women who referred to Qom's health centers, and the effect of ZnO nanoparticle on fungal agents.

In this laboratory-descriptive study was conducted on 150 patients susceptible to vaginitis. The samples were directly examined and cultured. The culturing was done on the Sabouraud Dextrose Agar medium containing Cloramphenicol, Corn Meal Agar medium including Tween80, Candida Crom agar medium and the test of germ tube and sugar assimilation test (API20C) to isolate and identify *Candida* species. Then the effect of different antifungal drugs and ZnO nanoparticles on them was examined by using disk diffusion method.

Among 150 samples examined, 83 cases (55.3%) were identified as having*Candida* species. The statistical analysis of isolated samples showed that *Candida albicans* (62.7%), *Candida krusei* (33.7%) and *Candida tropicalis* (%3.6) have the highestfrequency. Sensitivity pattern of these species to different antifungal drugs were different. Furthermore, ZnO nanoparticle showed a considerable no-growth halo diameter in some isolated *Candida* species, in comparison with other antifungal drugs. The research findings show that ZnO nanoparticle has antifungal effect and can be used as a proper alternative to remove*Candida* species in medical field.

Keywords: Candida Species, Antifungal Drugs, ZnO Nanoparticle

INTRODUCTION

Candidiasis is one of the most epidemic fungal infections in women, which is caused by different *Candida* species, especially *Candida albicans*. Today, this infection is considered the fourth common cause of hospital infections. The equivalent terms for Candidiasis are "Candidal Vaginitis", "Moniliasis" and "Aphtha (or thrush)"[Rajabibazl M, Javad Rasaee M, Nouri Fard M, Farahnejad Z. 2013]. This infection is observed on skin, nail, vaginal mucus, bronchus, lungs and gastrointestinal tract (digestive system) acutely, sub-acutely or chronically. It sometimes spreads and damages kidney, lungs, liver, heart, etc. The host's reactions to this disease range from a slight itch and inflammation to acute chronic septic form or Granulomatosis[Nozari S, Moghaddam AS, khoshdel A, Noorifard M, Moosavi AA. 2013]. The results of different studies show that the most common species causing Candidiasis is *Candida albicans* and 10-20 percent of other cases are caused by *Candida glabrata*, *Candida krusei* and *Candida parapsilosis* **[Nasrollahi Omran A, Vakili L, Jafarpur M 2010]**.

Most of *Candida* species are considered pathogenic in human. Some of thesemicroorganisms havetwo shapes and grow in the form of yeast, pseudo- mycelium and real mycelium. For example, *Candida glabrata* grows only in the form of yeast while *Candidaalbicans* forms a real mycelium on host's tissue. These microorganisms are found commensally in oral mucus, vagina, and gastrointestinal tract **[Falahati M, Sharifinia S, Foroumadi AR, Bolouri F, Akhlagh L, Yazdan Parast A, *et al*., 2009]**.

In recent years, the number of organisms resistant against antifungal drugs has increased and caused problems in treatment process. Nearly 10% of patients suffering from vaginal Candidiasis do not respond to initial treatment [Pádua RAFd, Guilhermetti E, Svidzinski TIE 2003]. This disease has been found and reported in all cities in Iran in different forms depending on the strength and weakness of immunity system **[Nozari S, Moghaddam AS, khoshdel A, Noorifard M, Moosavi AA. 2013]**.

Nanoparticles are the preliminary principles in nanotechnology. Among nanoparticles, non-organic metal oxides such as TiO_2, Cao, MgO and ZnO have a strong antimicrobial effect and have been taken into consideration[Oladiran AA, Olabisi IA-M. 2013]. The main mechanism of the effect of nanoparticles on microorganisms is that they

damage protein and DNA and destruct cell wall[Pasquet J, Chevalier Y, Couval E, Bouvier D, Noizet G, Morlière C, et al. 2013, Higa LH, Schilrreff P, Perez AP, Morilla MJ, Romero EL. 2013]. Among these mixtures, ZnO nanoparticle plays an important role in damaging microbic cell by generating free radicals. In *Escherichia coli* bacterium, these nanoparticles increase penetrability of membrane and destruction of cell and have inhibitory effect on such fungi as *Phozarium* **[Jehad M Y, Enas N D. 2012]**.

Given the ever increasing resistance of microorganisms, the present paper seeks to study the sensitivity of isolated *Candida* agents to drugs, report the results to health centers to help them prescribe drugs better, find proper ways to overcome these microorganisms, and observe the better effect of ZnO nanoparticle in comparison to other antifungal drugs.

MATERIALS AND METHODS

Sample Collecttion

This is a laboratory-descriptive study which was conducted by gynecologists on 150 patients susceptible to vaginitis in February and March 2014, using sterile swaps. The research population includes women susceptible to Candidal infection with clinical symptoms of vulvovaginitis and without these symptoms, who referred to hospitals in Qom province, Iran, including Izadi Hospital, ShahidBeheshti Hospital and Al-Zahra Hospital. Swaps were immediately transmitted into a tube containing one ml of physiological sterile serum and sent to laboratory.

Isolation of *Candida*Species

All samples were cultured on Sabouraud Dextrose Agar medium containing Cloramphenicol in order to isolate yeasts. Cycloheximide was not used due to sensitivity of some *Candida* species such as *Candida krusei, Candida parapsilosis* and *Candida tropicalis* to this antibiotic. The plates were incubated for 24-48 hours in 30^0C. Then a slide was made from produced colonies and studied in terms of their yeastiness, after the gram was stained **[Zaini F, Mehbod A, Emami M. 1999]**.

Identification of *Candida* Species

After the yeasts had grown, they were cultured on *Candida* Crom agar medium, using streak method, and incubated for 24 hours in 30^0C in order to isolate the samples including more than one type of yeast **[Hashemi S. 2011]**. Then one loop was taken from every stained colony and cultured on Corn Mill Agar medium containing Tween 80, through streak and linear culturing and pour plate and surface methods, and a steak was put between streak and linear cultures to

provide difficult conditions for Chlamido conidia and pseudo- mycelium to be produced. The plates were heated for 48 hours in 30^0C. Finally, the existence or non-existence of Chlamido conidia was examined by using weak lensing magnification of microscope **[Kurtzman C, Fell JW, Boekhout T. 2011]**. A part of yeast colony was transferred into a tube containing 0.5 ml of human serum and produced suspension was heated for 2-3 hours in 37^0C in order to examine germ tube formation. Then a drop of the suspension was put between slide and steak and examined by microscope **[Kelley D. 2006]**. The colonies which had produced green color on Candida Cromagarmedium and Chlamidoconidia on Corn Mill Agar medium containing Tween 80 in microscopic examination of colonies were considered as *Candida albicans*, the blue colonies which had produced long pseudo-mycelium and many blastopores along with pseudo-mycelium were considered as *Candida tropicalis* and those violet-pink colonies with jagged edges as *Candidakrusei* **[Hashemi S. 2011]**. Furthermore, sugar assimilation test (including glucose, maltose, sucrose, trehalose, cellobiose and raffinose) was conducted by using AP120C kit **[Evans EGV, Richardson MD. 1989]**.

AntibiogramTest

Using standard disk diffusion method, antibiogram test was conducted for 83 positive samples. These tests were conducted on Mueller Hinton Agar e medium which was inoculated by fungus suspension (equal to 0.5 McFarland). The plates were heated for 24 hours in 30^0C and the diameter of halos was interpreted with regard to the table presented by the factory that made the disks[Bauer A, Kirby W, Sherris JC, turck, Turck M. 1966]. The strain ATCC10231 of *Candidaalbicans* was used for positive control.

The Evaluation of the Effect of ZnO nanoparticle

Disk diffusion method and sterile paper disks moistened with 50 mg density were used to evaluate the effect of ZnO nanoparticle. First, 0.5 McFarland suspension of isolated species was densely cultured on Mueller Hinton Agar environment containing 2% glucose and 0.5 mcg/ ml of Methylene Blue and paper disks moistened with ZnO nanoparticle were put on the cultures by sterile forceps. Then plates were heated for 24 hours in 30^0C and the results were measured by ruler and recorded **[Jehad M Y, Enas N D. 2012]**.

Statistical Analysis

Charts were drawn by using Excel software. T-test was used to identify the significant difference between the effect of antifungal drugs and that of ZnO nanoparticle.

RESULTS

Isolation and Identification of *Candida* Species

150 patients susceptible to Candidal infection with clinical symptoms of vulvo vaginitis and without these symptoms were studied in this research, among whom 83 *Candida* species (55.3%) were isolated and 67 negative cases (44.7%) were reported.

As **Figure 1** shows, among 83 cases of isolated *Candida* species, 52 cases were identified as *Candida albicans* (62.7%), 28 cases as *Candida krusei* (33.7%) and 3 cases as *Candida tropicalis* (3.6%).

The Results of Antibiogram

Resistance and/or sensitivity of isolated *Candida* species to some antifungal drugs andZnO nanoparticle were examined, which showed that these species were resistant against some antifungal drugs and sensitive and semi-sensitive to some others. The resistance of isolated *Candida* species from patients against different antifungal drugs was different.

ZnO nanoparticle showed a considerable no-growth halo diameter in some isolated *Candida* species, in comparison with other antifungal drugs. Furthermore, ZnO nanoparticle had a significant difference, in comparison with Amphotricin B, Fluconazole, Ketoconazole, and Caspofungin ($p<0.05$).**Figure 2** shows resistance and sensitivity pattern of isolated *Candida* species to mentioned antifungal drugs.

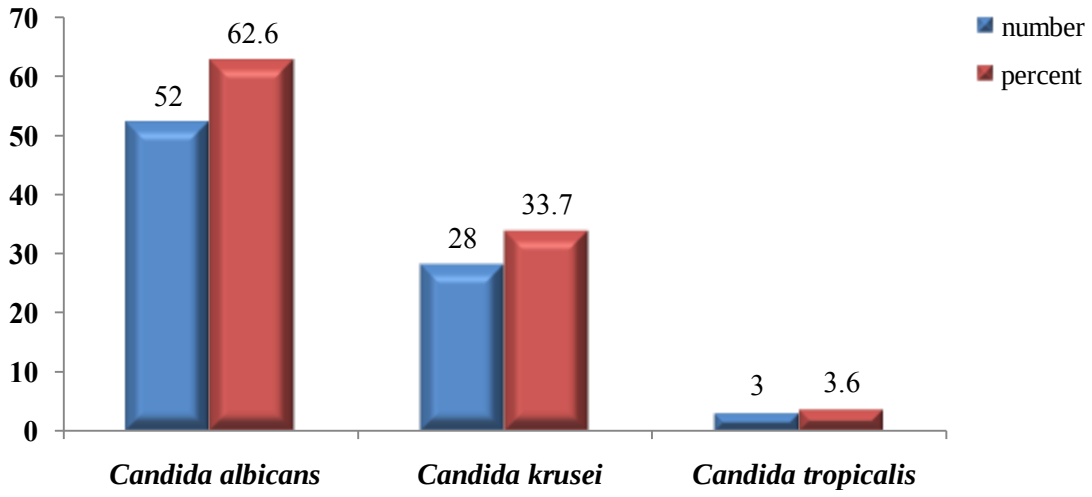

Figure 1: Distribution of number and percentage based on isolated yeast species

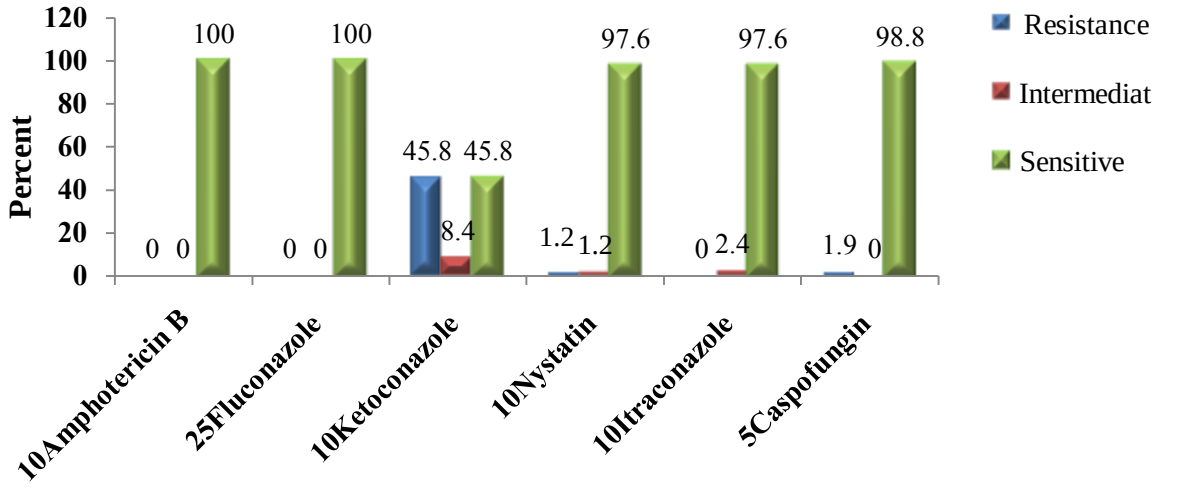

Figure 2: The resistance and sensitivity pattern of isolated *Candida* species to antifungal drugs

DISCUSSION

Given the growing epidemic of vaginitis infection among Iranian women (about 75%) and the difference between the pattern of yeast species and that of antibiogram in each district, the present paper seeks to examine these patterns in Qom province in order to identify causing factors and obtain their resistance pattern. Furthermore, it tries to provide physicians with proper pathological techniques, by using such new sciences as nanotechnology and ZnO nanoparticle. The findings showed that ZnO nanoparticle has antifungal effect and can be used as a proper alternative to remove *Candida* species in medical domain.

Having conducted necessary tests on 150 patients susceptible to *Candida*, 83 positive cases were found. Like most studies, *Candida albicans* had the highest frequency (62.7%) in this study. *Candida krusei* and *Candida tropicalis* constituted 33.7% and 3.6% of cases, respectively. In 2008, Falahati *et al* reported that 80 patients out of 150 patients suffering from vaginitis had Candidal vaginitis, with *Candida albicans* having the highest frequency and *Candida krusei* and *Candida gilermondi* the lowest frequency **[Falahati M, Sharifinia S, Foroumadi AR, Bolouri F, Akhlagh L, Yazdan Parast A, *et al*. 2009]. In 2011, Nazeri *et al* and Nasrollahi *et al.*,** found that *Candida albicans* and *Candida krusei* had the highest and lowest frequencies, respectively **[Nasrollahi omran A, Vakili L, Jafarpur M. 2011, Nazeri M, Mesdaghinia E, Moravej SAR, Atabakhshiyan R, Soleymani F 2012]**. In 2013, Panchal *et al*.

reported that out of 100 samples of vaginal swab 40 cases were positive, 22 of which were related to *Candida albicans* and 18 cases were non-Candidal **[Panchal P. 2013]**.

Different isolated *Candida* species showed different sensitivity to antifungal drugs. 45.8% of all *Candida* species was resistant against Ketoconazole and *Candida albicans* species was reported as the most resistant one. All *Candida* species isolated from patients were completely (100%) sensitive to Fluconazole and Amphotricin B. Although some studies show that the degree of sensitivity to Fluconazole is less than 100%, this difference may be due to difference in population under study or drug purity degree. Moreover, all *Candida* species showed 97.6% of sensitivity to Nystatin and Itraconazole and 98.8% to Caspofungin. In 2003, Padua *et al* examined antifungal activity of drugs on 400 secretory samples and reported that sensitivity pattern of isolates to Fluconazole, Nystatin and Amphotricin B was 96%, 71% and 98.8%, respectively **[Pádua RAFd, Guilhermetti E, Svidzinski TIE. 2003]**. In 2007, Pfaller *et al* examined 205329 fungus samples in 40 countries and reported that nearly 90.1% of all *Candida* species are sensitive to Fluconazole [**Pfaller M, Diekema D, Gibbs D, Newell V, Meis J, Gould I, *et al*. 2005]**. In 2007, Shirzi et.al examined the sensitivity of eight *Candida* species in 106 local isolates to Fluconazole and reported that 69.8% of isolates was sensitive to this antifungal drug **[Pakshir K, Akbarzadeh M, Bonyadpour B, Mohagheghzadeh AA. 2010]**. In 2009, Pakshir et.al found that 94% of all samples were sensitive to Clotrimazole, 55% to Fluconazole and 99% to Nystatin **[Pakshir K, Akbarzadeh M, Bonyadpour B, Mohagheghzadeh AA. 2010]**.

Furthermore, the results of this study showed that ZnO nanoparticle had antifungal effect and significant difference with other antifungal drugs such as Amphotricin B, Fluconazole, Ketoconazole, and Caspofungin ($p<0.05$) and showed a considerable no-growth halo diameter in some isolated *Candida* species. In 2011, Husseini et al. examined and compared the antifungal effect of ZnO nanoparticle on inhibiting the growth of standard strain of *Candida albicans* with that of Fluconazole. MIC limit for ZnO nanoparticle was 1.013-296 mg/ml and for Fluconazole 0.062-128 mg/ml **[Hosseini SS , Roudbar Mohammadi Sh , Joshaghani HR , Eskandari M 2011]**. This study showed that ZnO nanoparticle had antifungal effect and can be used as a proper alternative to remove *Candida* species. In 2013, Pasquet *et al*. introduced ZnO as a new preservative, with

effective antimicrobial activity on bacteria and fungi **[Pasquet J, Chevalier Y, Couval E, Bouvier D, Noizet G, Morlière C, *et al*. 2013]**. In 2012, Jehad *et al* examined antimicrobial activity of ZnO and ZnO nanoparticles on *Candida albicans* and reported that ZnO showed 10mm no-growth halo and ZnO nanoparticles showed 18mm no-growth halo, indicating that ZnO nanoparticles have stronger effect **[Ehad M Y, Enas N D. 2012]**. ZnO nanoparticles showed 12-40 mm no-growth halo diameter on isolated *Candida* species in this study.

CONCLUSION

The present study carry out to investigate the drug sensitivity of *Candida* agents isolated from the vaginal samples of women who referred to Qom's health centers, and the effect of ZnO nanoparticle on fungal agents. In this laboratory-descriptive study was conducted on 150 patients susceptible to vaginitis. The samples were directly examined and cultured Given the growing epidemic of vaginitis infection among Iranian women (about 75%) and the difference between the pattern of yeast species and that of antibiogram in each district, it is necessary to study these patterns in order to identify their causing factors and the pattern of resistance. Furthermore, using such new sciences as nanotechnology and ZnO nanoparticle will provide physicians with proper pathological techniques.

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