



THE EFFECT OF VAGINAL BACILLUS (LACTOBACILLUS ACIDOPHILUS) TOWARDS CANDIDA SPP. ISOLATED FROM WOMEN WITH CANDIDIASIS

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ABSTRACT

The aim of the current study was to investigate the isolation of vaginal *Lactobacilli* (*L. acidophilus*) and vaginal *Candida* from women complaining of vaginal candidiasis, as well as study the antagonistic effect between these microorganisms. *L. acidophilus* bacteria and vaginal *Candida* yeast were isolated from one hundred High Vaginal Swab .Samples from patients complaining candidiasis who consulted the obstetrics and gynecology consultant at two main hospitals in Mosul city. This study showed a significant prevalence of 76 yeast isolates 76%, out of a total of 100 HVS samples. The isolated *Candida* strains were further identified by the routine microbiological procedure as *C. albicans*, *C. femata*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. lusitaniae* isolated by proportions 53.94%, 23.68%, 10.53%, 5.26%, 1.33%, 2.63%, 2.63% respectively. Also the strains of *Lactobacillus* were further identified by the routine microbiological procedure and by API 50 CHL systems as *L. acidophilus* (53.4%), *L. gasseri* (22.7%), *L. vaginalis* (13.6%), *L. casei* (5.6%), and *L. crispatus* (4.4%). The results of the antagonism test indicated that vaginal *L. acidophilus* strains had no ability to inhibit *C. albicans*, the causative organism of vaginal candidiasis, but they showed a high ability to inhibit *C. femata*, and demonstrated clear inhibitory activity towards *C. glabrata*, and *C. tropicalis*. Also, they showed inhibitory capacity towards *C. parapsilosis*, but showed a lower inhibitory effect against *C. krusei* and *C. lusitaniae*. The causes and conditions leading to such results were discussed and some suggestions that may be due to the possibility of exploiting the phenomenon of antagonism between the two types of organisms have been set to reduce the incidence of vaginal candidiasis. In vitro antimicrobial activity of the vaginal lactobacilli (*L. acidophilus*) on *Candida*, suggesting that they could be a promising candidate for protection against vaginal candidiasis.

KEYWORDS: *Lactobacillus acidophilus*, Vaginal *Candida*, Candidiasis, Antagonism, Microbial growth inhibition.

INTRODUCTION

Microbial communities have an essential role in the health of humans and disease. Vaginal infection is the most health problem prevalent in women. Incompetent or bad diagnosis, wrong treatments, and resistance to antibiotic are the major causes of the failure of conventional antimicrobial treatment against these infections [Superti

F, De Seta F, 2020]. Vaginal candidiasis is common globally among many women. The most frequent etiologic agent is *Candida albicans* [Samaka HM et al., 2018]. So when disrupted the balance of the vaginal microbiota, that leading to facilitated overgrowth of *Candida* spp. Pregnancy, susceptibility, antibiotic therapy, use of spermicide and contraceptives, frequent sexual intercourse, immunosuppression, and diabetes are factors that increase the risk for candidiasis development [Sobel DJ, 2007]. The lack of H₂O₂-producing *Lactobacillus* species have been associated with the development of vaginal candidiasis [Vitali B et al.,

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2007]. The lactobacilli capacity to adhere and compete for the adhesion sites on vaginal mucosa can be contributed for inhibition to the colonization of Candida [Verdenelli MC et al., 2014].

Lactobacilli have an important role in the prevention of vaginal infections. Several studies have also found that the strains of lactobacillus can displace *C. albicans* from the epithelial cells of vagina [Gil NF et al., 2010; Kumherová M et al., 2021]. The several antimicrobial products produced by Lactobacilli also prevent vaginal-colonization by other different pathogens [Raz-zak MS et al., 2011]. The Lactobacilli also produce hydrogen peroxide (H_2O_2) which is found to inhibit the growth of several pathogens [Matu MN et al., 2010].

In addition to the lactic acid bacteria in the vagina, which have been proven beyond a reasonable doubt, that help in maintaining the natural balance of the vagina, there are pathological neighborhoods that settle in the vagina, but without waiting for the opportunity, including Candida (especially *C. albicans*) coexisting and causing vaginal infection [Barnett JA, 2008].

The common cause of the symptomatic episodes of vaginal candidiasis is *C. albicans*. It is responsible for 20- 85% of vaginal candidiasis [Lines A et al., 2020]. Although *C. albicans* is from normal flora in the mouth and digestive and vaginal quantities in small quantities and does not cause any disease when present with bacteria that inhibit their growth, however, in the absence of bacteria (such as lactobacilli), Candida gets adhesion to epithelial cells and will multiply and become able to injury events in different regions of the host

[Figueiredo Carvalho MGH et al., 2017]. The success of these opportunistic fungi in causing disease may be due to their heat tolerance at 37°C and their ability to change their shape.

Plants are known sources of natural medicines. Several plants show significant anti-Candida activities and some of them have

lower minimum inhibitory concentration, making them promising candidates for antiCandida therapy. However, none of these plant products is marketed for anti-Candida therapy because of lack of sufficient information about their efficacy, toxicity, and kinetics. Soliman S and co-authors reported the major plants that have been tested for anti-Candida activities with recommendations for further use of some of these plants for more investigation and in vivo testing including the use of nanostructure lipid system [Soliman S et al., 2017].

The objective of this study was to investigate vaginal Lactobacilli (*L. acidophilus*) and vaginal Candida from women with vaginal candidiasis, as well as study of the antagonistic effect between these microorganisms.

MATERIAL AND METHODS

SPECIMEN COLLECTION AND ETHICAL CLEARANCE

One hundred 100 high vaginal swab (HVS) specimens were collected from the patients who consulted the Obstetrics and Gynecology Consultant at Al-Khansaa and Al-Batool Hospitals in Mosul city, aged between 20 and 72 years. After completing a form prepared for this purpose, it reflects their health and social condition. Vaginal smears were taken from the patients suffering from clinical symptoms of vaginitis from the top of the vagina in collaboration with the doctor specialized in the use of colposcope vaginal speculum device sterile conditions [Forney LJ et al., 2010]. The following direct tests were carried out on samples taken to support the initial clinical diagnosis.

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Verbal and writing consents were taken from each patient before directing the study. The protocol of the study and the subject information and consent form were reviewed and approved by a local committee of ethics (Mosul Health directorate, Iraq), under reference No. BMS/0358/016.

ISOLATION OF LACTOBACILLI

The isolation of Lactobacilli was performed by the microbiological procedure and inoculation on a solid medium using MRS (de Man Rogosa and Sharpe) agar plates as a selective media for the Lactobacilli isolation, were incubated in an anaerobic jar in the presence of a Gas Generating Kit at



To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

37 °C for 24-48 h [Ayenalem S et al., 2010]. Before discarding them as negative, the plates were re-incubated for further overnight, after 24 h incubation on MRS agar Gram stain, and morphology was examined [Aslim B, Kilic E, 2006]. Analytic Profil Index API test used in order to confirm the diagnosis of isolates. Isolation of *Candida albicans* To isolate *C. albicans* using Sabouraud-dextrose agar plates as a selective media for *Candida* isolation were incubated at 37° C for 24-48 h, then Gram stain and morphology were examined. In order to confirm the diagnosis of isolates, the *Candida* API system was used. *C. albicans* yeast was diagnosed with Germ Tube Formation Test [Forbes BA et al., 2007]. The test was carried out to distinguish *C. albicans* yeast and the newly described yeast *C. dubliniensis* from the other types of yeast. 0.5 ml of human serum was placed in each of the sterile test tubes. Each tube was inoculated with a small portion of growing colonies on the SDA medium and incubated at a temperature of 35-37° C for no more than three hours, then took one drop of the suspension and placed on a clean glass slide and then covered with the cover slide and examined under the microscope at a magnification force (40X) to observe the presence of sprouting cells, which resembles in the form of a hand mirror.

IN VITRO DETERMINATION OF ANTAGONISTIC ACTIVITY OF VAGINAL LACTOBACILLI AGAINST CANDIDA

Seven vaginal candida isolates were used to study the inhibitory efficacy of *L. acidophilus* bacteria, the test was conducted using the Agar well diffusion method is also known as Hole plate diffusion method [Brantner B et al., 1994]. Method of spreading with Muller Hinton Agar 0.1 ml of

vaginal *Candida* isolates to be tested, which contains 10 cells/ml, leaving the medium for minutes to soak, and then drilling was done in the middle with a diameter 5 mm using sterile cork perforator and add to each hole a small drop from the sterile and cooled MRS agar medium at a temperature of 47° C and leave to solidify in the holes and add to each hole 0.1 ml of the young bacterial culture of the *L. acidophilus*, which contains 10 cell /ml and dishes incubation under ideal conditions for a period of 24-48 hours.

After incubation, the activity of antimicrobial was determined by measuring the inhibition zone diameter around the wells and followed to conduct the antimicrobial test between vaginal *Candida* and lactobacilli, the test was conducted by Muller Hinton agar inoculated with 0.1 ml of the young bacterial culture of lactobacilli (*L. acidophilus*) containing 10 cells/ml, leaving the medium for minutes to obtain impregnation, and then drilling was done in the middle with a diameter of 5 mm using a sterile cork perforator and add to each hole a small drop from the center of the sterile and cooled dextrose at a temperature of 47°C and left to solidify in thepits and add to each hole 0.1 ml of young vaginal *Candida* isolates to be tested which contain 10 cells/ ml and incubated dishes under ideal conditions for a period of 24-48 hours, after incubation, inhibition areas were observed and diameters were measured.

RESULTS AND DISCUSSION

A number of vaginal *Candida* species were isolated and identified, which gave clear results regarding germ tube formation, which is one of the impor-

TABLE 1.

Recovery rates and Characterization of vaginal *Candida* yeast strains

Vaginal Candida Isolation	NO.	Growth on Sabouraud agar	Growth on Sabouraud agar with chloramphenicol	Growth on Blood agar	Germ Tub formation	%
<i>C. albicans</i>	41	+	+	+	+	53.94
<i>C. glabrata</i>	8	+	+	+	-	10.53
<i>C. tropicalis</i>	4	+	+	+	-	5.26
<i>C. parapsilosis</i>	1	+	+	+	-	1.33
<i>C. krusei</i>	2	+	+	+	-	2.63
<i>C. femata</i>	18	+	+	+		23.68
<i>C. lusitaniae</i>	2	+	+	+		2.63
Total	76					100

TABLE 2.

Recovery rates and Characterization of vaginal *Lactobacillus* strains

Vaginal Lactobacilli Isolation	NO.	Growth on MRS agar	Growth on Blood agar	%
<i>L.acidophilus</i>	47	+	+	53.40
<i>L. gasseri</i>	20	+	+	22.72
<i>L. vaginalis</i>	12	+	+	13.63
<i>L. casei</i>	5	+	+	5.68
<i>L. crispatus</i>	4	+	+	4.57
Total	88			100

tant tests to diagnose *C. albicans*. Table 1 illustrates the characteristics of studied isolates. Microscopic examination of *Candida* cells was confirmed using gram stain, as well as a wet preparation examination of the models. Based on diagnostic results *C. albicans* 41(53.94%), *C. femata* 18 (23.68%), *C. glabrata* 8(10.53%), *C. tropicalis* 4 (5.26%), *C. parapsilosis* 2 (1.33%), *C. krusei* 2 (2.63%), and *C. lusitaniae* 2 (63%) were chosen to study the antagonism with the lactic acid bacteria (*L. acidophilus*).

As for the lactobacilli, It has been confirmed from their characteristics matching to the genus *Lactobacillus*. A total of 88 (88%) *Lactobacillus* strains were isolated from 100 HVS samples in this study. The *Lactobacillus* strains were identified by API 50 CHL systems, as *Lactobacillus acidophilus* 47 (53.40%), *Lactobacillus gasseri* 20 (22.72%),

Lactobacillus vaginalis 12 (13.63%), *Lactobacillus casei* 5 (5.68%), and *Lactobacillus crispatus* 4(4.57%) (Table 2). Isolates of lactic acid bacteria were obtained but *Lactobacillus acidophilus* was used only due to its high effectiveness in antimicrobial against microorganisms.

The antimicrobial activity of the vaginal lactobacilli with *Candida albicans* showed that this yeast was not affected by the *L. acidophilus*, (Figure 1). But *L. acidophilus* bacteria showed high inhibitory activity against the second type of *Candida* (*C. femata*) isolated in this study, and the inhibition zone diameter was 16 mm. and demonstrate clear inhibitory activity towards *C. glabrata* and *C. tropicalis*, where the inhibition zone diameter was (12,10) mm, and showed inhibitory capacity towards *C. parapsilosis* 9mm, while they showed a lower inhibitory effect against both *C. krusei* and *C. lusitaniae*; the diameters of the inhibition areas was 7 mm (Table 3).

The result of antagonism test showed that *C. albicans* yeast was not affected by the *L. acidophilus*, and this result was compatible with the result obtained by [Itapary dos Santos C et al., 2019] who evaluated the activity of antagonistic of vaginal *Lactobacillus* spp. on *C. albicans* and they showed that 5 out of 20 *Lactobacillus* strains were did not presented any inhibitory effect on *C. albicans*. However, it was found [Wang S et al., 2017] found that vaginal *Lactobacillus* was able to inhibit the growth, formation of hypha, and also regulate the virulence-related gene expressions in *C. albicans*.

Regarding the antimicrobial phenomenon of *L. acidophilus* towards *C. albicans*, results found that the lactobacilli failed to affect *Candida* that it

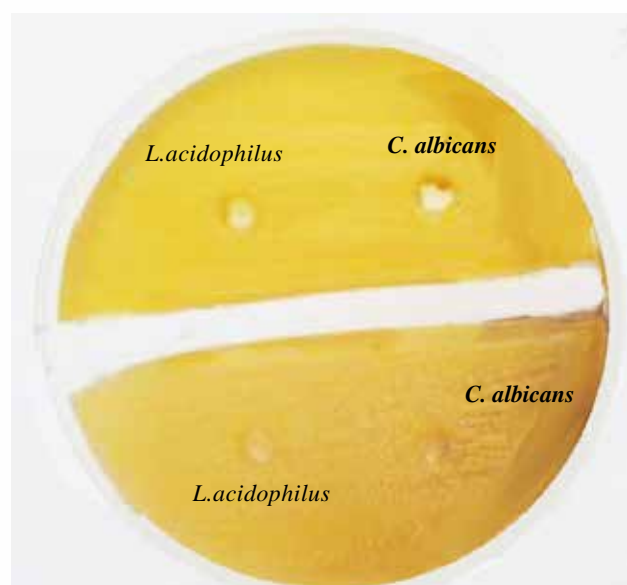


FIGURE 1. Growth inhibitory effect of Vaginal Bacillus (*Lactobacillus acidophilus*) against *Candida*.

TABLE 3.

The antagonism effect of *L. acidophilus* bacteria towards vaginal *Candida* yeast

<i>Candida</i> spp.	Inhibition zone Diameter (mm)
<i>Candida albicans</i>	0
<i>C.femata</i>	16
<i>C. glabrata</i>	12
<i>C.tropicalis</i>	10
<i>C.parapsilosis</i>	9
<i>C.krusei</i>	7
<i>C.vaginalis</i>	7

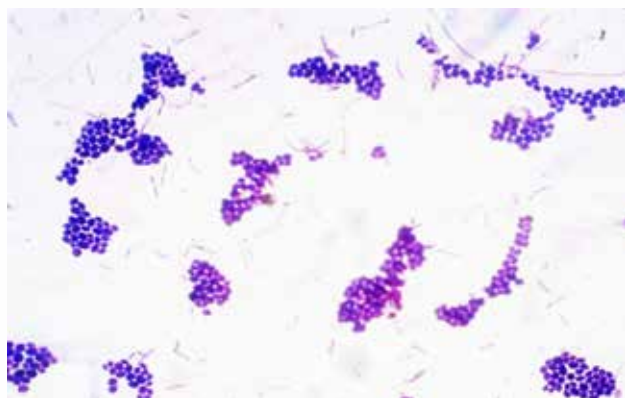


FIGURE 2. Vaginal *Lactobacillus* and *Candida albicans* growing on blood agar

shared and coexist with in the vaginal environment as confirmed by (Figure 1, 2), which indicates synergy without contrast between the two organisms. This result was compatible with Dasari and his colleagues [Dasari S et al., 2014] who found that the lactobacilli antimicrobial activity is little towards fungi and yeasts.

This inhibitory activity of *L. acidophilus* bacteria towards the vaginal candida isolates was studied (*C. femata*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. vaginalis*) as in (Figure 3) due to certain inhibitory substances include metabolites like organic acids [Zárate G, Nader-Macias ME, 2006], diacetyl, hydrogen peroxide [Geria M et al., 2014], acetoin, 2,3-butanediol, acetaldehyde, bacteriolytic enzymes, benzoate, bac-



FIGURE 3. Growth inhibitory effect of Vaginal *Lactobacillus acidophilus* towards 1. *C. femata* 2. *C. glabrata* 3. *C. tropicalis* 4. *C. parapsilosis* 5. *C. krusei* 6. *C. vaginalis*

teriocin, reuterin, etc. [Kaewsrichan J et al., 2006] display antagonistic activity towards many pathogenic microorganisms.

Itapary dos Santos and his colleagues [Itapary dos Santos C et al., 2019] investigated the antagonistic effect of vaginal *Lactobacillus* spp. on *C. albicans* to verify whether active compounds of *Lactobacillus* spp. had the antifungal, and they found that 15 out of 20 strains of *Lactobacillus* had inhibitory activity on *C. albicans*.

Zang and his colleagues [Zang I et al., 2020] reported that there is a triple interaction between the host, species of *Lactobacillus*, and *Candida* with regard to the fungal infections and the potential antifungal and fungistatic activity of *Lactobacillus* species. They also revealed that these interactions are incomplete due to the variability of the involved isolates and species and the varying complexity of the human host.

Studies have also investigated the biosurfactant (BS) isolated from vaginal *Lactobacillus crispatus* strain, *L. crispatus* BC1, and its safety and anti-adhesive/antimicrobial activity against *Candida* spp., and they revealed that BS from vaginal *L. crispatus* BC1 is able to interfere with *Candida* adhesion in vitro and in vivo, and suggest its potential as a preventive agent to reduce mucosal damage occasioned by *Candida* during vulvovaginal candidiasis [De Gregorio PR et al., 2020; Abruzzo A et al., 2021]. So, the result of our study was compatible with other studies [De Gregorio PR et al., 2019; Parolin C et al., 2021; Russo R et al., 2019] which found that the lactobacilli showed in vivo and in vitro antimicrobial activity against *Candida*.

CONCLUSION

The effect of prokaryotic microorganism *L. acidophilus* may be absent from other microorganisms somewhat distant from it, such as yeasts (*C. albicans*), which represent Eukaryotes. A main potential application of the current study concerns the effect *Lactobacillus* (*L. acidophilus*) to propose as probiotics for the prophylaxis and/or the adjuvant therapy of vaginal candidiasis. Future studies are encouraged to evaluate the technological properties of those microorganisms for clinical use, including determination of their stability and viability in pharmaceutical preparations such as intravaginal administration capsules.

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