A STUDY ON THE ANTIFUNGAL EFFECTS OF LACTOBACILLUS SPP. ON CANDIDA

Nagla Abdel Moniem Radi[*], Abeer Ahmed Abdelmonem[*] and Adel Ahmed Ziada [**]

**Department of Medical Microbiology and Immunology, Faculty of medicine, BeniSuef University*

***Department of Clinical Pathology, Faculty of medicine, Al Azhar University*

ABSTRACT

Background: *Yeast infections represent one of the most important hospital acquired infections, Candida infection is the commonest one. The use of Probiotics in prevention and treatment of different bacterial infections reduces the risk of emerging resistant bacterial strains to antibiotics that commonly used.* ***Objectives:*** *This study investigates the antifungal effect of Lactobacillus spp. on Candida isolated from blood cultures.* ***Methodology:*** *Ten Lactobacilli strains were isolated from the children stool using de Man, Rogosa and Sharpe (MRS) agar and biochemically identified by api® 50 chl medium. Fifty Candida strains were isolated from blood cultures using Sabouraud Dextrose (SD) agar. The test of antifungal effects of Lactobacilli strains on Candida were done by using plate well diffusion technique on SD agar.* ***Results:*** *The Lactobacillus acidophilus had the most effective antifungal effect against Candida albicans and non-albicans.* ***Conclusion****: the results of this study will help to find out new anti-fungal agents against resistance problem. Therefore, further and more detailed studies are needed in this field.*

Keywords: *Antifungal effect, Lactobacillus, Candida, Blood culture*

Corresponding author:

Dr. Nagla Abdel Moniem Radi

Telephone No. *0201004434264*

Mail: naglaradi@yahoo.com

INTRODUCTION

Fungi are microorganisms that broadly found in nature. They are present in a human body as normal flora in the intestinal system, mouth etc. Hospital-acquired fungal infections may cause dangerous morbidity and mortality, so the fungal diseases are one of the important public health problems[1].

Candida spp., accounted for more than 80% of hospital-acquired yeast infections. Recently the significant increase in *Candida* non-*albicans* species, especially from blood cultures indicates that *C. albicans* and non-*albicans* species have a high risk of infection in the systemic blood circulation[2]. Immunosuppressed individuals, transplant recipients, low birth weight neonates, and patients under chemotherapy are more susceptible to invasive diseases caused by Candida, most often as bloodstream infections (candidaemia) with a risk to disseminate to different organs, such as the liver, spleen, bones, and heart[10]

Lactic acid bacteria (LAB) have been reported to produce antimicrobial substances that inhibit growth of pathogenic and saprophytic microorganisms. Other compounds like organic acids, hydrogen peroxide also included in their antimicrobial effects[3]. Recent many studies concerning on the antifungal effects of *Lactobacillus* strains. Focused on treatment and prevention of uro-genital candidiasis and fungal food contamination[4,5].

Atanasova et al[6] approved that *Lactobacillus paracasei* subsp. *Paracasei* M3 bacterium had an anti-fungal effect on *C. albicans*, *C. blankii and C. pseudointermedia*. Resistance of *Candida* species to different antifungal agents is increasing especially in hospital acquired infections[7]. Despite the established new antifungal agents, it is very important to study antifungal effect of probiotics like *Lactobacillus* on *Candida* isolated from blood cultures.

THE AIM OF THE PRESENT STUDY

Is to study the antifungal effect of human intestinal *Lactobacillus* spp. on *Candida* yeasts isolated from blood cultures and the pattern of *Lactobacillus* spp. that commonly used as antifungal probiotic in Egypt.

METHODOLOGY

Microorganisms:

-Ten *Lactobacillus* bacterial spp. were isolated from stool samples of children (5-10 years old) who attended the Pediatric Outpatient Clinics, Beni- Suef University Hospital, without complaining from any gastrointestinal diseases and no history of antibiotics taken in the last two weeks.

- All samples had been examined and processed at the Microbiology

Laboratory of the Faculty of Medicine, Beni- Suef University.

- The isolates of *Lactobacilli* spp. were kept in tubes at -20 ^{0}C until used.
- .-*Lactobacilli* were identified using *api® 50 chl* biochemical system. (BioMérieux's, France).
- Blood samples were collected from patients with clinical signs of septicemia or bacteremia from ICU, Newborn Units, and Cardiology Units at Beni-Suef University Hospital.
- The collected Bottles of blood cultures were incubated for 7 days at 37C^O. The bottles which had growth positive were sub-cultured on to Sabouraud dextrose agar plates.
- Each *Candida* strain was isolated from each sample was tested for germ tube formation to differentiate *candida albicans* from non-albicans spp, then stored in eppendorf tubes at -20 ^{0}C[8].

Antifungal Assay:

The anti-fungal effect of *Lactobacillus* spp. on *Candida* strains was tested using well agar diffusion technique[9]. *Lactobacilli* were inoculated on MRS liquid culture medium (Oxoid, USA) under anaerobic conditions at 37 ^{0}C for 24-48 h. The cultured fluid was centrifuged at 4.000 rpm for 10 min. The supernatant was filtered through 0.45 µm pore filter (Millipore, Molsheim, France) [9]. *Candida* spp. were cultured on SDA and adjusted to 0.5 McFarland turbidity standards. In all cultured media, 10 mm diameter wells were opened by the cork borer and 100 µl filtered *Lactobacillus* supernatant were added. The plates were kept at room temperature for 2 h then incubated at 37 ^{0}C for 48 h. Finally, the zones formed around the wells were measured in mm, compared with that of control which contained MRS broth only. **The diameter more than 6 mm was considered positive[9].**

RESULTS

The distributions of 50 isolates of *Candida* isolated from blood cultures were 23 out of 50 (46%) from ICU, 12(24%) from Newborn Unites, and15 (30%) from Cardiology Units (table 1).

Table (1): the distribution of *Candida* strains isolated from blood cultures

ICU		**Newborn units**		**Cardiology units**	
***Candida* strains= 23 (46%)**		***Candida* strains= 12 (24%)**		***Candida* strains= 15 (30%)**	
C. albicans	***C, non -albicans***	***C. albicans***	***C, non -albicans***	***C. albicans***	***C, non -albicans***
6	**17**	**2**	**10**	**3**	**12**

The types of isolated strains of *Candida* were 11 (22%) out of 50 *C. albicans* and 39 (78%) were non-*albicans* species. Our study shows that the prevalence of non-*albicans* species is higher than that of *C. albicans* that indicate the importance of non-*albicans* species in the pathogenicity of hospital acquired blood stream infections in Egypt.

The isolated *Lactobacilli* strains were 6 out of 10 (60%) *Lactobacillus acidophilus*, 2 (20%) were *Lactobacillus casei* , one (10%) was *Lactobacillus rhamnosus* and *Lactobacillus plantarum* was 1 out of 10 (10%). The results shown in table 2.

Table (2): The types of isolated lactobacilli strains

Total		***L.acidophilus***	L.***casei***	***L.rhamnosus***	***L.plantarum***
No	**10**	**6**	**2**	**1**	**1**
%	**100%**	**60%**	**20%**	**10%**	**10%**

Table 3: shows that *Lactobacillus acidophilus* had anti-fungal effect against *10 out of 11 (91%) of C. albicans* and 17 0ut of 39 (43.5%) of non-*albicans* strains with mean diameter of inhibitory zone of both 13 mm. However, the effect of *Lactobacillus rhamnosus* against *Candida albicans* was 6 out of 11 (54.5%) and non-*albicans* 11out of 39 (28%) with mean diameter of inhibitory zone 10 mm , while *Lactobacillus casei* anti-fungal effect, against *Candida albicans* was 2 out of 11 (18%) and non-*albicans* 5 out of 39 (13%), with mean diameter of inhibitory zone 8 mm and *Lactobacillus plantarum* anti-fungal effect against *Candida albicans* was 1 out of 11 (9%) with the diameter of inhibitory zone 6.5 mm but no antifungal effect was noticed against non-*albicans.*

Tab. (3): The anti-fungal effects of *Lactobacillus* on isolated *Candida* species

	L .acidophilus						*L. casie*						*L. rhamnosus*						*L. plantarum*					
The inhibitory effects	***C.albicans***			***C.*non *albicans***			***C.albicans***			***C.*non *albicans***			***C.albicans***			***C.*non *albicans***			***C.albicans***			***C.*non *albicans***		
	N	**T**	**%**	**N**	**T**	**%**	**N**	**T**	**%**	**N**	**T**	**%**	**N**	**T**	**%**	**N**	**T**	**%**	**N**	**T**	**%**	**N**	**T**	**%**
	10	**11**	**91**	**17**	**39**	**43**	**2**	**11**	**18**	**5**	**39**	**13**	**6**	**11**	**54**	**11**	**39**	**28**	**1**	**11**	**9**	**0**	**0**	**0**

The zone of inhibition	***c.alb.***	**C.non *alb.***	**mean**	***C.alb.***	**C.non *alb.***	**mean**	***C.alb.***	**C.non *alb.***	**mean**	***C.alb*.**	**C.non *alb.***	**mean**
	15	**11**	**13**	**9**	**7**	**8**	**11**	**9.5**	**10**	**6.5**	**0**	**6.5**

Lactobacillus acidophilus was seen to have the most effective anti-fungal effects against both *albicans and non-albicans* (67.5%) followed by *Lactobacillus rhamnosus* (41%) and *Lactobacillus casei* (15.5%), However *Lactobacillus plantarum* had the least anti-fungal effects (9%).

DISCUSSION

Different studies showed that some protein molecules produced by certain bacteria have anti-fungal effect[6]. **Özkaya et al**.[11]studied the anti-fungal effect of *Lactobacillus* on various fungi. It was observed that *Lactobacillus* bacteria had varying degrees of antifungal effects against *Candida albicans* and non-*albicans* strains. A study of *L. plantarum* and *C. albicans* mixed cultures under anaerobic conditions at 37 ^{0}C for 48 h showed growth in *L. plantarum*, but not in *C. albicans*[12]. It was found that *L.rhamnosus* and *L.acidophilus* probiotic bacteria isolated from human stools had a highly effective anti-fungal effect against *C. albicans*[13]. In our study, *Lactobacillus acidophilus* had the highest anti-fungal effect.

A study by **Matsubara et al.** [14] showed the mechanism of how *Lactobacillus* species may inhibit *C. albicans* host colonization. These data clarified the inhibitory mechanisms of probiotic candicidal activity of *Lactobacilli*, thus supporting their therapeutic mode against candidal infections of the mucosa.

There are few studies focus on the anti-fungal effects of *Lactobacillus* originating from human intestinal system on yeasts (*Candida*) isolated from the human blood circulation system. Our study could be considered the newest one in this respect. Presence of yeasts in the blood circulation system in children and immune-compromised patients has a great risk. Recently, the appearance of resistance against anti-fungal drugs represents a major health problem. *Lactobacillus* spp. of human origin, which has probiotic effects, may be used against fungus through their anti-fungal effects, especially if combined with anti-fungal drugs.

In conclusion, the results of this study will help to find out new anti-fungal agents against resistance problem. Therefore, further and more detailed studies are needed in this field.

REFERENCES

1. **Chen KW, Chen YC, Lo HJ, Odds FC, Wang TH, Lin CY, Li SY:** Multi locus sequence typing for analyses of clonality of *Candida albicans* strains in Taiwan. *J Clin Microbiol*, 44, 2171- 2178, 2006.
2. **Levy I, Rubin LG, Vasistha S, Tucci V, Sood SK:** Emergence of *Candida parapsilosis*as the predominant species causing Candidemia in children. *Clin Infect Dis*, 26, 1086-1088, 1998.
3. **Sezer Ç, Güven A:** Investigation of bacteriocin production capability of lactic acid bacteria isolated from foods. *Kafk as UnivVetFakDerg*, 15, 45-50, 2009.
4. **Corsetti , A, Gobbetti M, Rossi J, Damiani P:** Antimould activity of sourdough lactic acid bacteria: Identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco*CB1. *Appl Microbiol Biotechnol*, 50, 253-256, 1998.
5. **Barousse MM, van der Pol BJ, Fortenberry D, Orr D, Fidel Jr PL:** Vaginal yeast colonization, prevalence of vaginitis, and associated local immunity in adolescents. *Sex Transm Infect*, 80, 48-53, 2004.
6. **Atanassova M, Choiset Y, Dalgalarrondo M, Chobert JM, Dousset X, Ivanova I, Haertle T:** Isolation and partial biochemical characterization of proteinaceous anti - bacteria and anti –yeast compound produced by *Lactobacillus paracasei* subsp. *Paracasei*

strain M3. *I J Food Microbiol,* 87, 63-73, 2003.

7. **Berry V, Badyal DK:** Sensitivity of clinical isolates of *Candida* species to antifungal drugs. *J Med Educati on Res,* 8, 214-216, 2006.
8. **Warren NG, Hazen KC:** Cryptococcus and other yeasts of medical importance. In *Manual of Clinical Microbiology,* ed. Murray, P.R., Baron, E.J.O., Tenover, F.C. and Yolken, R.H. pp. 1184- 1199. Washington: ASM Pres, 1999.
9. **Toba T, Samant SK, Itoh T:** Assay system for detecting bacteriocin in micro-dilution wells. *LettApplMicrobiol,* 13, 102-104, 1991.
10. Arendrup MC: Epidemiology of invasive candidiasis. Curr Opin Crit Care 16(5):445–452. doi:10.1097/MCC.0b013e32833e84d2, 2010
11. **Özkaya FD, Karabıçak N, Kayalı R, Esen B:** Inhibition of yeasts isolated from traditional Turkish cheeses by *Lactobacillus* spp. *IntJDairyTechnol,* 58, 111-114, 2005.
12. **Wynne AG, McCartney AL, Brostoff J, Hudspith BN, Gibson GR:** An in-vitro assessment of the effects of broad-spectrum anti biotics on the human gut microflora and concomitant isolation of a *Lactobacillus plantarum* with anti-Candida activities. *Anaerobe,* 10, 165-169, 2004.
13. **Verdenelli MC, Ghelfi F, Silvi S, Orpianesi C, Cecchini C, Cresci A:** Probioti c properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faces. *Eur J Nutr,* 48, 355-363, 2009
14. **Matsubara VH, Wang Y,Bandara HM, Mayer MP:** J Appl. Microbio. Biotechnol.Jul;100(14):6415-26, 2016