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ORIGINAL ARTICLE

A study on the Antifungal Effects of *Lactobacillus* spp. on Candida Species

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ABSTRACT

Background: *Yeast infections represents one of the most important hospital acquired infections. The use of Probiotics in prevention and treatment of different bacterial infections reduce the risk of emerging resistant bacterial strains to antibiotics that commonly used.* ***Objective:*** *This study investigates the antifungal effect of Lactobacillus on candida isolated from blood cultures.* ***Methodology****: Ten Lactobacilli strains were isolated from the children stool using MRS agar and biochemically identified by API 50CHL. Fifty Candida strains were isolated from blood cultures using Sabouraud dextrose agar. The test of antifungal effect of Lactobacilli strains on Candida was done by using plate well diffusion technique on SD agar.* ***Results:*** *The Lactobacillus acidophilus had the most effective antifungal effect on C. albicans and non albicans.*

INTRODUCTION

Fungi are microorganisms that are 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. constitutes 80% of hospital-acquired yeast infections. There has been a significant increase in *Candida non-albicans* species, especially in blood cultures indicating that *C. albicans* and non-albicans pose a high risk of infection in the systemic blood circulation [2] There is a growing interest in probiotic bacteria to prevent and combat fungal diseases[3].

Probiotic bacteria are defined by the World Health Organization as 'Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[4]. The most common probiotic genera Bifidobacterium and Lactobacillus are believed to act by competitive action on pathogens from different sites, by competing for available nutrients, and by altering the mucosal immune host defense [5] Lactic Acid Bacilli (LAB) has been reported to produce antimicrobial substances that inhibit growth of pathogenic and saprophytic microorganisms. Other compounds like organic acids and hydrogen peroxide are also included in these antimicrobial effects[6]. Many studies are exploring the antifungal effects of *Lactobacillus* strains and their role in treatment and prevention of urogenital candidiasis and fungal food contamination[7,8]. Atanasova et al.[9] 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[10]. Despite the established new antifungal agents, it is very important to study the 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 on Candida yeasts isolated from blood cultures and the pattern of *Lactobacillus* spp. that commonly used as antifungals in Egypt.

METHODOLOGY

Microorganisms:

- Ten *Lactobacillus* bacterial spp. were isolated from the stools of children 5-18 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 Microbiological Laboratory of the Faculty of Medicine, Beni- Suef University.
- Culture of all samples was done on MRS medium obtained from (Oxoid Company). The medium is a selective medium for isolation of *lactobacillus* and inhibits accompanying microflora. The medium contains polysorbate, acetate, magnesium and manganese which are known to act as special growth factors for *lactobaciili* as well as a rich nutrient base. Most of the accompanying microflora can be inhibited by thallium acetate, sorbic acid, acetic acid, sodium nitrite, cycloheximide and polymyxin.

- The plates were inoculated under aerobic and anaerobic condition at 37º C for 24hours and 48 hours respectively and colonies were examined.

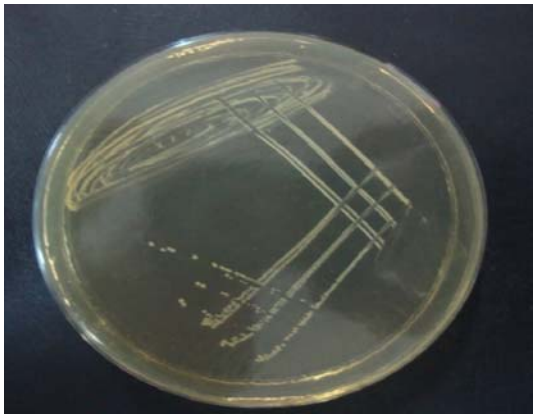

Fig. 1: MRS agar medium inoculated with lactobacillus

The growth of lactobacillus was identified by:

- *Gram stain:* Gram positive bacilli, rode shape, non capsulated, non spore forming with branched, chain or non specific arrangement
- *Catalase test: lactobacilli* are catalase negative.
  - The isolated *Lactobacilli* spp. were kept in tubes at -20 ^{0}C until used.

Lactobacilli were identified using API 50 CHL biochemical system. (BioMérieux's, France).

Fig. 2: API readings of *lactobacillus rhamnosus*

- Seventy two 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 37CO. Positive blood culture bottles were subcultured on to Sabouraud Dextrose Agar plates.
- Each Candida strain isolated from each blood samples was tested for germ tube formation and Chlamydospore production to differentiate between candida albicans and non albicans spp[10]

Germ tube test

- Small portion of an isolated colony of the yeast to be tested is suspended in a test tube containing 0.5 ml of human serum.
- The test tube is incubated at 35˚C for no longer than 2 hours.
- A drop of the yeast-serum suspension is placed on microscopic slide, overlaid with a coverslip, and examined microscopically for the presence of germ tubes

Chlamydospore production

By using Rice Extract Agar medium (REA) **.**This medium was used for chlamydospore production to differentiate between *candida* species.

It consists of: Rice 20 g, Distilled water litre, Agar 20 g.

The rice was cooked in the water for 45 minutes and after filtration the agar was added, the medium was autoclaved and poured into petri dishes.

Inoculation on Rice Extract Agar (REA) medium:

- Three parallel cuts about 5 mm apart into agar were made with holding the inoculating wire at about a 45 angle.
- A coverslip was laid on the surface of the agar covering a portion of the inoculation streaks.
- The inoculated plates were incubated at 30˚C for 24 to 48 hours and examined microscopically through the coverslip.
- The Rice Extract Agar preparation was examined for the presence of chlamydoconidia . The isolates were stored in bead tubes at -70 ^{0}C[11].

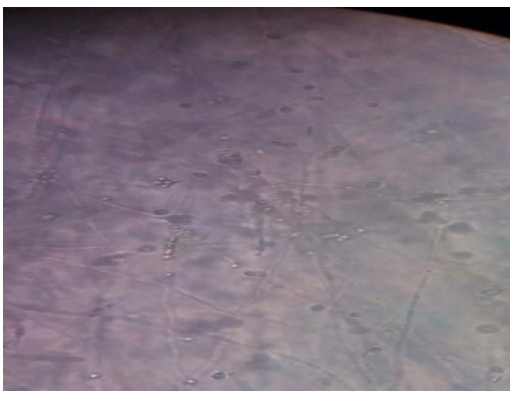

Fig. 3: Candida albican chlamydospore production

Anti-fungal Assay:

Anti-fungal effect of *Lactobacillus* spp. on *Candida* strains was examined using agar well diffusion technique[12]. *Lactobacilli* were inoculated on MRS liquid culture medium (Oxoid, USA) under anaerobic conditions at 37 ^{0}C for 24-48 h. The cultured fluid were centrifuged at 10.000 colony forming unit for 10 min. The supernatant was filtered through 0.45 µ mill pore

filter (Millipore, Molsheim, France)[12]. *Candida* spp were cultured on SDA. All SDA cultured plates, 10 mm wells were opened by the cork borer and 100 µl filtered *Lactobacillus* supernatant were added. The plates were kept at room temperature for 2 hours then were 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[12].

RESULTS

- 50 Candida stains isolated from 72 blood samples (69.4%)
- The distribution of the isolates were 23 out of 50 (46%) from ICU, 12 (24%) from Newborn Unit, and 15 (35%) from Cardiology Unit (table 1).

Table 1: The distributions of *Candida* strains isolated from blood cultures

ICU		**Newborn units**		**Cardiology units**	
***Candida* strains= 23 (46%)**		***Candida* strains= 12 (24%)**		***Candida* strains= 15 (35%)**	
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 spp.* were 11 (22%) of 50 *C. albicans* and 39 (78%) non-albicans species. Our study shows that the pathogenicity of non-albicans species is much higher than that of *C. albicans* which indicates the important role of non-albicans species in the hospital acquired blood stream infections in Beni - Suef.

The isolated lactobacilli strains were 6out of 10 (60%) *lactobacillus acidophilus* , 2 (20%) were *lactobacillus casei* ,one strain (10%)was *lactobacillus rhamnosus* and *lactobacillus plantarum* was 1 out of 10 (10%) the result is shown in table 2.

Table 2: The isolated species of lactobacilli

l.acidophilus		***L. casei***		***L. rhamnosus***		***l plantarum***	
NO	%	NO	%	NO	%	NO	%
6	60%	2	20%	1	10%	1	10%

Table 3 and Fig 4 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 13mm. 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 10mm , 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 8mm and *Lactobacillus plantarum* anti-fungal effect against *Candida albicans* was 1 out of 11 (9%) with the diameter of inhibitory zone 6.5mm but no antifungal effect was noticed against *Candida non albicans.*

Table 3: The anti-fungal effect of *Lactobacillus* on isolated *candida* species

	L.acidophilus						***L. casei***						***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.albicans**		**c.non albicans**		**mean**		**c.albicans**		**c.non albicans**		**mean**		**c.albicans**		**c.non albicans**		**mean**		**c.albicans**		**c.non albicans**		**mean**	
	15		**11**		**13**		**9**		**7**		**8**		**11**		**9.5**		**10**		**6.5**		**0**		**6.5**	

n: the number of candida strains with inhibitory zone more than 6mm

Lactobacillus acidophilus was seen to have the highest anti-fungal effect on both *Candida albicans and Candida non albicans* (67.5%), followed by *Lactobacillus rhamnosus* (41%) and *Lactobacillus*

casei (15.5%) while *Lactobacillus plantarum* had the least anti-fungal effects (9%).

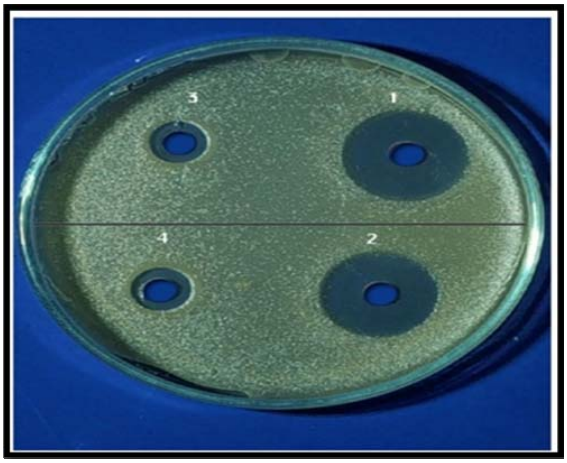

Fig. 4: The antifungal effects of *Lactobacillus spp* on *Candida albicans*

*1- *Lactobacillus acidophilus*
*2- *Lactobacillus rhamnosus*
*3- *Lactobacillus casei*
*4- *Lactobacillus plantarum*

DISCUSSION

Different studies showed that some protein molecules produced by certain bacteria have anti-fungal effect[6]. Özkayaet al.[17] 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°C for 48 h showed a growth of *L. plantarum*, but not *C. albicans* which indicates the inhibitory effects of *L. plantarum* on *C. albican s*[18]. 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*[19]. In our study, *Lactobacillus acidophilus* had the highest anti-fungal effect.

These data clarified the inhibitory mechanisms of probiotic candicidic activity of *lactobacilli*, thus supporting their therapeutic mode against candidal infections of the mucosa.

Matsubara et al[12] clarified The inhibitory effects of the probiotic Lactobacillus on C. albicans entailed both cell-cell interactions through reducing the early stages of Candida biofilm formation and secretion of exometabolites that may impact on pathogenic attributes associated with C. albicans colonization on host surfaces and yeast filamentation.

There are few studies focus on the anti-fungal effects of *Lactobacillus* originating from human intestinal system on yeasts isolated from the human blood circulation system. Our study could be considered the newest one in this respect. The presence of yeasts in blood circulation system in children and immunocompromised patients has a great risk as 50 candida ssp isolated from 72 blood sample (69.4%). 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[12].

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.

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