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Full Length Research Paper

Dramatic scenario of urogenital *Candida albicans* in presence of Lactic acid bacteria (LAB)

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Yeast infections of the vagina are more prevalence and frequently recurring problem of women. Such infections are caused primarily by *Candida albicans* and apparently occur when the environmental balance in the-vagina is disturbed. *Lactobacillus acidopbilu*s restricts the growth of pathogenic microorganisms per se displayingwide inhibitory spectrum substances. Fifteen (30%) *C. albicans* isolates were obtained through 50 vaginal swabs from patients who visited the obstetrics and Gynecology clinic departments of hospitals in Baghdad city during the period of March to July 2010. Local isolates of *L. acidophilus* was evaluated as antifungal activity against vaginal isolates of *C. albicans*. Such activity was comprised with common four antifungal agents (Nystatine, Fluconazol, Griseofulvin and Amphotricin B). Susceptibility of *C. albicans* isolates to *L. acidophilus* bacteria were determined by means of disc diffusion assay. Results of susceptibility were showed *in-vitro* that all isolates 15 (100%) sensitive to Nystatine after cultivation on ESDA agar at 37°C for 24 h. Sensitivity of *C. albicans* to *L. acidophilus* suspension was less than Nystaine but still high with 13 sensitive isolates (86.6%). Fluconazol and Griseofulvin antifungal agents were showed same susceptibility of 10 sensitive isolates (66.6%) against *C. albicans* growth, while Amphotricin B had relatively low sensitivity with 7 isolates (46.5%). All results of susceptibility are being detected by the importance of *L. acidophilus* bacteria in re equilibrium normal flora of vagina that prevent or reduce the chances of vaginal infection especially recurrent vaginal candidiasis (RVC) cases. Also the findings of the study suggest that using of yoghurt as local application can be an important role to reduce or prevent RVC cases, and this can be verify by continuous efforts to make sure of probiotic effects of lactobacilli species.

Key words: *Lactobacillus acidophilus, Candida albicans*, vaginitis.

INTRODUCTION

The three common types of acute vaginitis are bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis (Aisner and Murillo, 1989). Recurrent vaginal candidiasis (RVC) can be defined as the occurrence of at least four mycological proven symptomatic episodes within 12 months , or at least three episodes unrelated to antibiotic therapy within 1 year (Andreu, 2004; Al-Amery, 2005). Two main hypotheses have been proposed for the causes of recurrent vaginitis (i) reinfection through sexual transmission or from reservoirs in other organ system (Gastrointestinal or Urinary tracts) (Al-Shibly, 2006); (ii) relapse due to incomplete eradication of *Candida* spp. that have increased virulence or are drug-resistant (Baron et al., 1994; Buckly, 1989). Decreased cell-mediated local immunity is also a risk factor for RVC (Al-Shibly, 2006). *Lactobacilli* are both predominant bacteria in the vaginal tract and the regulator of normal vaginal flora. *Lactobacilli* make lactic acid, which maintains the normal vaginal pH between 3.8 to 4.5, and inhibit the adherence of bacteria to vaginal epithelial cells (Aisner and Murillo, 1989).

Opportunistic yeasts that belong to the genus *Candida* are causative agents of urogenital infections especially vaginal candidiasis. About 75% of women would have at least one episode of vaginal infection during their lifetime (Carr et al., 2002); 15-20% of women without any specific predisposing factor will experience repeated infections within 3 months of treatments (Casals, 1997).

C. albicans is the dominant species that causes vaginitis, with a prevalence of 70 to 90% in various

reports, while up to 20 to 30% in non-albicans species have been reported to be associated with RVC cases (Collee et al., 1996).

Approximately 60% of urogenital lactobacilli strains produce hydrogen peroxide, which inhibits the growth of bacteria and destroys HIV *in-vitro* (De Man et al., 1960). Estrogen improves lactobacilli colonization by enhancing vaginal epithelial-cell production of glycogen, which breaks down into glucose and acts as a substrate for the bacteria (Aisner and Murillo, 1989).

Although lactobacilli are the dominant bacteria, others also present in the urogenital or vagina, including *Streptococcal* Spp., Gram-negative bacteria, *Gardneralla vaginalis*, and anaerobes. *C. albicans* can also be found in normal flora as a commensally agent in 10-25% of asymptomatic women (Eckert et al., 1998).

The study was amid to indicate the activity of *L. acidophilus* (LAB) *in-vitro* against *C. albicans* in urogenital site or reducing the chances (ability) to cause RVC in women's patients, that also explaining or meaning recreation or re equilibrium of candidian urogenitalflora.

MATERIALS AND METHODS

Specimens' collection

Fifty vaginal swabs were collected from women who suffering from recurrent vaginal candidiasis, that diagnosed through specialist physicians of obstetrics and Gynecology clinic departments of many hospitals in Baghdad city during the period of March to July 2010.The common criteria of patients included in this study were complaint of RVC cases. These patients included women from wide age range 17-50 years old, and many of them were pregnant.

Vaginal swabs were collected aseptically, transported immediately in test tube containing normal slain (0.9%) to laboratory (Emmons et al., 1974). These swabs were cultured on different media for isolation and identification of *Candida* ssp.

Yeast isolates:

All vaginal swabs cultured at 37°C for 72 h on:

1. Christenses Urea Agar (CUA) as primary media for isolation of yeast colony from pathogenic samples (Faro, 1996).
2. Sabouraud's Dextrose Agar (SDA) for isolation and diagnosis of yeast samples (plates) (Ferrer, 2000).
3. Emmon's Sabouraud's Dextrose Agar (ESDA) for diagnosis of Candida species from positive candida cultures (Ferrer, 2000).

To complete the diagnosis of positive candida cultures as *C. albicans* many tests were carried out (Fidel and Sobel, 1996; Forbes et al., 2002) which included:

1. Macroscopic appearance (colony morphology) of candida colonies on CUA, SDA, and ESDA medium for shape, size, color and odor of colonies.
2. Microscopic appearance (Gram stain method) to select carried out to study the hyphae and buds.
3. Chlamydospers formations, germ tube formation, growth on 37°C, were also done (Gould and Bowi, 1982; Gunston and Fairbrother, 1975).
4. Sugars fermentation tests include Galactose, Glucose, Trehalose, Maltose, lactose and sucrose according method described by Hawes et al. (1996).
5. Sugars assimilation tests include Sucrose, Maltose, Glucose, Lactose, Melibiose, Xylose, Trehalose, Galactose, Inositol and Cellobiose was carried according to method described by Faro (1996).
6. Confirmation of isolates were done by using API 20C AUX and API 32C (bio merieux) profile (Hindler, 1998).

The indication of yeast isolates had done in bacteriological laboratory of Institute of Medical Technology -Baghdad, Iraq.

Lactobacillus isolates

Lactobacillus acidophilus local isolate was obtained from stock culture (of yoghurt) collections of food science and Biological Technology Department, Agriculture College of Baghdad University. This isolate was re cultured on De Man Rogosa Sharpe agar (MRS), incubated anaerobically (at 5-10%CO_2) at 37°C for 24 h (Holt et al., 1994).

Confirmation tests were done (Holt et al., 1994) such as:

1. Macroscopic appearance of cultured colonies on MRS agar for its shape, color and viscosity of colonies.
2. Microscopic appearances of colonies after Gram stain (shape of cells, sporulation and capsule formation).
3. Biochemical tests(John et al., 2008) for pure isolates were done like, Oxidase , Gelatinase , Catalase, Growth on MRS agar at 45°C, Production of Ammonia from Arginine, and Production of acid and curd on litmus Milk agar .
4. Carbohydrates fermentation to Galactose, Glucose, lactose, Trehalose, Manitol, Zylol, Sucrose, Fructose, Mannose, Arbinose and Rafinose (Kaewsrichan et al., 2006).

L. acidophilus suspension was done to be used in susceptibility test with concentration 1% from liquid culture of bacteria which contain $1x10^8$ cells/ml (Kandler et al., 1986). *C. albicans* suspension also was done by using 1 ml of yeast inoculate that contain $1x10^5$ cells/ml calculated by hemacytometer (Klebanoff and Coombs, 1991).

Stock solution of antifungal Nystatin, Fluconazol, Griseofulvin and Amphotricin B were done with concentration of $1x10^4$ µg/ml of solution to be used in susceptibility test according to Klebanoff and Coombs (1991), whereas filter paper discs were done from *L. acidophilus* bacterial suspension and solution of the four antifungal agents (Lanchares and Hernandez, 2000).

Susceptibility test

Susceptibility to *L. acidophilus* and four listed antifungal agents were determined against *C. albicans* isolates by means of a disc diffusion assay as described in Handle procedure (Linda and Echert, 2006), then recording the inhibition zones which formed around each agents on ESDA agar. The study was taking up 14 mm and above zone of inhibition just positive.

Statistical analysis

Analyses of the data obtained from these variations were performed by measuring percentage and means value according to Ringdahl (2000).

RESULTS

Fifty vaginal swabs were collected of investigated RVC

Table 1. Results of characteristic tests of *C. albicans* isolates.

Physiological test	Reac.	Sugars ferment.	Reac.	Sugars assim.	Reac.
Chlamydospares formation	+	Galactose	+	Sucrose	+
		Glucose	+	Maltose	+
Germ tube formation	+	Trehalose	±	Glucose	+
Growth on 37°C	+	Maltose	+	Lactose	±
Capsule formation	-	Lactose	+	Melibiose	-
Urease production	-	Sucrose	-	Xylose	+
				Trehalose	+
				Galactose	+
				Inositol	-
				Cellobiose	-

Table 2. Results of biochemical and carbohydrates fermentation of *L. acidophilus* isolates.

Biochemical tests	React.	Carbohydrates fermentation	React.
Oxidase	-	Galactose	+
Gelatinase	-	Glucose	+
Catalase	-	Lactose	+
Growth on 45°C	-	Trehalose	+
Production of ammonia	-	Manitol	+
Production of acid	-	Zylol	+
		Sucrose	+
		Fructose	+
		Manose	+
		Arbinose	+
		Rafiuose	+

patients during the course of this study, there were only 39 cases (78%) showed positive yeast culture. After culturing of positive samples on CUA, SDA and ESDA medium there were 15 isolates of *C. albicans* (30%) were obtained from total clinical swabs and (26%) of positive plates which had only yeast episodes of RVC. *Candida* spp. on culture media were showed white creamy , smooth , homogenous colonies as macroscopic appearance , while microscopic appearance showed Gram positive staining cocci, budded cells with pseudohyphae. Other features of diagnosis of *C. albican* sisolates were listed in Table 1, which showed the characteristic reactions of physiological tests, sugars fermentation tests and sugars assimilation. All results of API – Candida technique was confirmed the 15 *C. albicans* isolates.

Results of confirmation of local isolate of *L. acidophilus* was showed oval, white to yellowish color , with smooth border, non-bright and convex colonies. Under micro-scope the colonies were showed Gram positive, cocci, arranged with single, or pair to chains, non-spore forming cells. Also the colonies were showed negative reaction to Oxidase, Gelatinase, Catalase, and Growth on MRS agar at 45°C and production of ammonia from arginine, except production of acid and curd on litmus milk agar had positive reaction of biochemical tests. Results of carbohydrates fermentation of isolates had positive reaction (Table 2).

Susceptibility tests

The comparative *in-vitro* susceptibility of *C. albicans* isolates to the activity of *L. acidophilus* suspension and four selective effective antifungal agents (Nystatine, Fluconazol, Griseofulvin and Amphotricin B) are shown in Table 3. Fifty isolates (100%) of Candida were susceptible (sensitive) to Nystatine, this highly activity to inhibit the growth of all isolates comes from its polyenes compounds that integrating directly to sterol and ergosterol which found in the plasmic membrane of Candida cells to form pores , that lead to gradually changing in the cell permeability and death of these cells[36]. The results of susceptibility of *C. albicans* isolates were showed 13 isolates (86.6%) sensitive to the suspension of *L. acidophilus* and this results insure the

Table 3. Comparative *in-vitro* susceptibilities of *C. albicans* isolates from RVC cases.

Isolate No.	*L. acidophilus*	Nystatine	Fluconazol	Griseofulvin	Amphotricin B
1	+	+	+	+	-
2	+	+	+	+	+
3	+	+	+	+	-
4	+	+	-	-	-
5	+	+	+	+	+
6	-	+	-	-	-
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	-	-	-
10	+	+	+	+	+
11	-	+	-	-	-
12	+	+	+	+	+
13	+	+	+	+	+
14	+	+	-	-	-
15	+	+	+	+	-
Total	13 (86.6%)	15 (100%)	10 (66.6%)	10 (66.6%)	7 (46.6%)

(+) = sensitive, (-) = resistant.

highly activity of lactobacilli to inhibits the *Candida* growth. Thus, *L. acidophilus* bacteria would be useful agent (compound) for development of antifungal or antibacterial activity against vagina pathogens and has great potential for use in external application and vaginal wash for preventing or reducing *Candida* growth or might also treatment of RVC casesafter *in-vivo* testing and toxicological study of this agent.

Fluconazol and Griseoflvin were showed same results of susceptibility with 10 isolated (66.6%) to other agents that used. Also the results reported that *C. albicans* isolates were showed moderate resistance to Amphotricin B agent, 8 isolates (53.4%) (Table 3).

DISCUSSION

Results of fifty vaginal swabs that were collected and investigated as RVC cases during the course of this study showed there were only 39 cases (78%) positive yeast culture (Lanchares and Hernandez, 2000). While other clinical specimens were either negative culture or might be cases of bacterial infections or Trichomonas vaginosis (Wilson, 2004), however, episodes due to non-albicans species of Candida appear to be increasing (Ferrer, 2000). Understanding the changes that occur in patient's population which exhibit higher prevalence of non-albicans species is warranted (that is, menopausal and postmenopausal women and diabetic patients). Possible explanation includes changes in patient physiology, hormone balance, and decrease in immune function (John et al., 2008).

Isolation and diagnosis results showed that there were 15 isolates of *C. albicans* (30%) were obtained from total clinical swabs and (26%) of positive plates which had only yeast episodes of RVC, these results were agree with (Nyirjesy et al., 1995) and local study (Iraqi) (Majeed, 2004), who reported that the prevalence of candidiasis vaginitis were (38.5%) in Iraqi women patients after culturing the causative agents and diagnosis. In addition Ostrzenski (1974) reported the prevalence of C*. albicans* isolates were ranged between 35.1 to 39% in his samples of yeast isolated from the infected vagina of symptomatic women. Also macroscopic and microscopic features of *C. albicans* were indicated (Al-Amery, 2005) as shown in Table 1 showed the characteristic reactions of physiological tests, sugars fermentation tests and sugars assimilation. All results of API – Candida technique confirmed the 15 *C. albicans* isolates (Al-Shibly, 2006).

Results of confirmation of local isolate of *L. acidophilus* was showed oval, white to yellowish color, with smooth border non-bright and convex colonies (De Man et al., 1960). Under microscope the colonies were showed Gram positive, cocci, arranged with single, or pair to chains, non-spore forming cells (Kandler et al., 1986). Also the colonies were showed negative reaction to Oxidase, Gelatinase, Catalase, Growth on MRS agar at 45°C and production of ammonia from arginine, except production of acid and curd on litmus milk agar had positive reaction of biochemical tests (Holt et al., 1994; Kandler et al., 1986). Results of carbohydrates fermentation of isolates had positive reaction (Table 2) (Carr et al., 2002).

Susceptibility tests

Results of susceptibility shown in Table 3, reported that

fifty isolates (100%) of *Candida* were susceptible (sensitive) to Nystatine, this highly activity to inhibit the growth of all isolates comes from its polyenes compounds that integrating directly to sterol and ergosterol which found in the plasmic membrane of Candida cells to form pores, that lead to gradually changing in the cell permeability and death of these cells (Ostrzenski, 1974). Also these results were agreement to (Al-Amery, 2005; Al-Shibly, 2006) finding of local two studies.

The study used *L. acidophilus* as an example of lactic acid bacteria (LAB) as a comparison with other common antifungal agents, because we believe the ability of lactobacilli to inhibit the growth of Candida cells. Ostrzenski (1974) treated patients with vaginitis cases by a lyophilized culture of *L. acidophilus* suspended in cocoa butter, while Gunston and Fairbrother (1975) treated other patients with yoghurt. Each study gave inconclusive evidence that *Lactobacilli* were beneficial in treatment of vaginitis cases.

Many studies are certain that *Lactobacilli* are natural habitants of urogenital microflora and are believed to have a central role in the suppression of potential pathogens. *Lactobacilli* administered to the genital tract have a prominent role as a prophylactic aimed at improving the genital microflora defense against bacterial infection (Kaewsrichan et al., 2006). Also Wilson (2004) reported that *Lactobacilli* probiotic strains that can be used for the treatment of vaginal candidiosis should be able to produce metabolites that are fungi static for *C. albicans*.

Fluconazol and Griseoflvin were showed same results of susceptibility with 10 isolated (66.6%) to other agents that used, the study believe that this sensitivity of *C. albicaus* was not because of their efficiency but due to many patients (investigated) were received repeated courses of antifungal medication to deal with recurrent fungal infections or might these antifungal agents are often prescribed without confirmation of the diagnosis of vaginal infection by culture.

But Mohanty et al. (2007) showed that there was a low prevalence of fluconazol resistance in vaginal candida isolates in their population. Also these results agree with the finding of Okeke and Gugnany (1987), which showed that the Griseofulvin antifungal agent had narrow effects on some candida species.*C. albicans* isolates were showed moderate resistance to Amphotricin B agent , 8 isolates (53.4%) , that may occur due to wide usage in many infections which develop the resistance to the agent and reduce its activity on *Candida* isolates (Casals, 1997) specially those isolated from RVC cases (Al-Shibly, 2006).

Our results believe that poor vaginal hygiene, lack of standards of care, and insufficient attention paid to preventive urogenital health care is well known in nursing home patients, and improved vaginal care may reduce the occurrence of RVC cases and resistance to different effective antifungal agents.

All results of susceptibility are being detected the importance of *L. acidophilus* bacteria to maintain the balance of the vaginal flora and its protective and probiotic role in treating that preventing vaginal infection by producing antagonizing compounds which are hydrogen peroxide, lactic acid and bacteriocins (Hawes et al., 1996).

These properties of Lactobacilli lead to use these bacteria as probiotic agent in addition to being member of normal flora and are generally regarded as safe for use in humans. So that in treatment of vaginitis, the normal flora disturbances must be restored by avoiding the use of wrong antibiotic those disturb the normal vaginal flora (Andreu, 2004).

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REFERENCES

Aisner J, Murillo J (1989). Invasive mycosis in acute leukemia. J. Ann. Med., 8: 12-16.

Andreu A (2004). Lactobacillus as probiotic for preventing urogenital infection. Rev. Med. Microbiol., 15: 1-6.

Al-Amery NO (2005). A study of Taxonomy and Epidemiology of pulmonary mycotic infections in Al-Qadisyia province. Ph.D. thesis. College of Education. University of Al-Qadisyia.2005.

Al-Shibly MK (2006). Study of some Bio-properties and Histopathogical effects of some clinical isolates of *Candida Spp.* In Al-Qadisiya governorate. Ph.D. thesis. College of Education. Al-Qadisiya University. pp. 63.

Baron F, Beterson L, Finegold S (1994). Diagnostic Microbiology 9th ed. St. Louis. London.

Buckly HR (1989). Identification of yeast in Evans EG, Richard MD. Medical Mycology: A practical approach. IRL. Press. Oxford Univ. Press 1989.

Carr FJ, Chill D, Maida N (2002). The lactic acid bacteria: A literature survey. Crit. Rev. Microbial., 28: 281-370.

Casals FL (1997). Effect of Candidiasis on some immunological and histological characters in mice. Mycopathologia, 91: 22-30.

Collee JG, Fraser AG, Marmion BP, Simmons A (1996). Practical Medical Microbiology. 4th ed. Churchill Livingstone. UK.

De Man JC, Rogosa M, Sharpe ME (1960). A medium for the cultivation of *Lactobacilli*. J. Appl. Bact., 23: 130-135.

Eckert LO, Hawes SE, Stevens CE, Koutsky LA, Eschenbach DA. Holmes KK (1998). Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm. Obstet. Gynecol., 92: 757-765.

Emmons CW, Binford CH, Utz JP (1974). Candidiasis. In Medical Mycology. Lea and Fibiger. 2nd ed. Philadelphia.

Faro S (1996). Vaginitis: diagnosis and management. Int. J. Menopausal Stud., 41: 115-123.

Ferrer J (2000). Vaginal Candidiosis: epidemiological and etiological factors. Int. J. Gynecol. Obstet., 71: S21- 27.

Fidel PL, Sobel JD (1996). Immunopathogensis of recurrent Vulvovaginal candidiasis. Clin. Microbiol. Rev., 9: 335-338. (Abstract)

Forbes BA, Saham DF, Weissfeld AS (2002). Diagnostic Microbiology. 10th ed. Mosby. Inc. USA.

Gould JC, Bowi JI (1982). The determination of bacterial sensitivity to antibiotics. Edinburg Med. J., 59: 178-182.

Gunston KD, Fairbrother PF (1975). Treatment of vaginal discharge with yoghurt. So. Afr. Med. J., 49: 675-681.

Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-

Hanssen, Holmes KK (1996). Hydrogen peroxide-producing *Lactobacilli* and acquisition of vaginal infection. J. Infect. Dis., 174: 1058-1063.

Hindler J (1998). Antimicrobial susceptibility testing in: Essential procedures for clinical microbiologypress, Washington. pp. 207-248.

Holt JG, Krieg MR, Sneath P. HA, Staley JT, Williams ST (1994) Bergey's manual of determinative bacteriology. 9th ed. William and Wilkins, Baltimore.

John – Paul V, Matthew JS, Sean GC, Jason PT, Martin EA, Eli M, Scott EG (2008). Survey of Vaginal – Flora *Candida* Species isolates from women of different age groups by use of species – specific PCR detection. J. Clinc. Microbiol., 46: 1501-1503.

Kaewsrichan J, Peeyananjarassri K, Kongprasertkit J (2006). Selection and identification of anaerobic *Lactobacilli* producing inhibitory compounds against vaginal pathogens. FEMS Immunol. Med. Microbiol., 48: 75-83.

Kandler O, Weiss N. Genus (1986). *Lactobacillus* In: Berge's manual of systematic bacteriology. (Sneath P. HA, Mair NS. and Hol JG. ed. Vol. 2 Williams and Wilkins Co., Baltimore. M. D. USA.

Klebanoff SJ, Coombs RW (1991). Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. J. Exp. Med., 174: 289-292.

Lanchares JL, Hernandez ML (2000). Recurrent vaginal candidiasis changes in etiopathogenical patterns. Int. J. Gynaecol. Obstet., 71(Suppl. 1), S29-35.

Linda O, Echert MD (2006). Acute Vulvovaginitis. N. Engl. J. Med., 355: 12.

Majeed HA (2004). Diagnostic and Immunological study of *Candida* spp. caused vaginitis. MSc thesis. College of Education. University of Baghdad.

Mohanty S, Xess I, Hasan F, Kapil A, Mittal S, Tolosa JE (2007). Prevalence and susceptibility to fluconazole of *Candida* Spp. causing vulvovaginitis. Indian J. Med. Res., 126: 216-219.

Nyirjesy P, Seeney SM, Grody MH, Jordan CA, Buckley HR (1995). Chronic fungal vaginitis: the value of cultures. Am. J. Obstet. Gynecol., 173: 820-823.

Okeke CN, Gugnany HC (1987). *In-vitro* sensitivity of environmental pathogenic dermatiaceous fungi to Azole compounds and a Phenylpropyl Morpholine derivative. Mycopathology, 99:175-181.

Ostrzenski A (1974). Lyophilized suspension of *Lact. acidophilus* in supportive treatment of mycotic forms of vaginitis in women. Pol. Tyg. Lek., 30: 925.

Ringdahl EN (2000). Treatment of recurrent Vulvovaginal candidiasis. Am. Fam. Phys., 61: 3306-3312, 3317.

Wilson J (2004). Managing recurrent bacterial vaginosis. Sex Transm. Infect., 80: 8-11.