



# IN VITRO DETERMINING THE EFFECT OF VAGINAL LACTOBACILLUS ON SOME PATHOGENIC OF UROGENITAL INFECTIONS IN WOMEN

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## Abstract

It has been postulated that lactobacillus play a critical role in maintaining the normal vaginal ecosystem by preventing overgrowth the of pathogens and other opportunistic organisms by producing lactic acid hydrogen peroxide, bacteriocines and other antimicrobial substances. Current study aimed to determine the effect of vaginal lactobacillus *spp.* (*L. acidophilus*, *L. crispatus*, *L. gasseri* and *L. iners*), as probiotics isolated from vaginal swabs of fifty healthy women for reduction of urogenital infections (*Candida albicans*, *Gardenella vaginalis*, *Staphylococcus aureus* and *Escherichia coli*) isolated from female patients who attended the obstetric and gynecology clinics in Ibn-AL Baladi Hospital. The effects of lactobacillus strains supernatants agents urogenital pathogens were tested in agar plate diffusion method on Manns-Regoz and Sharpe (MRS) agar and in liquid medium (MRS broth). Also Minimum inhibitory concentration (MIC), Minimum bacteriocidal concentration (MBC) and Minimum fungicidal concentration (MFC) were determined. The result of antimicrobial activity of vaginal lactobacillus revealed that *L. crispatus* had the highest effect on urogenital pathogens in both methods while the result of MIC, MBC and MFC showed that the concentration 60% led to minimized growth of *G. vaginalis*, *S. aureus* and *E. coli* and concentration 70% inhibit growth of these bacteria completely, whereas sharp decrease in *Candida albicans* growth noticed in 80% concentration even as the concentration 90% and above inhibited all pathogenic isolates (MBC and MFC). From above results it be clear that all *Lactobacillus* strains have antimicrobial activity against urogenital pathogens but *L. crispatus* had the highest affect than others with promising inhibitory spectrum.

**Key words:** MIC, MBC, MFC, Vaginal lactobacillus, urogenital infections.

## Introduction

Studies about vaginal microflora indicated that microorganisms normally present in human vagina play a key role in preventing successful colonization by undesirable organisms including those responsible for bacterial vaginitis, yeast infections, sexually transmitted diseases and urinary tract infections (Vitali *et al.*, 2007). Clearly an accurate understanding of the composition and ecology of the vaginal microbial ecosystem in normal healthy women is essential in acquiring these communicable diseases (Nyirjesy *et al.*, 1997). The vagina and its unique micrflora form a finely balanced ecosystem, with the vaginal environment controlling the microbial types present and the microflora in turn controlling the vaginal environment (Atlas *et al.*, 1995)

*Lactobacilli* have been thought to be the predominant members of normal post pubertal vaginal microflora (Chenoll *et al.*, 2006). Unfortunately, these have not proven to be very successful, this could be because about 10%-40% of women whose vaginal microbial communities lack appreciable number of *Lactobacilli* apparently maintain normal vaginal ecosystems (Nyirjesy *et al.*, 1997). Therefore it need approach that enable to determine the numbers of lactobacilli actually preserve normal vaginal ecosystems. Agar plug diffusion and Agar well diffusion methods are widely used to evaluate the antimicrobial activity of microbial extracts, also dilution methods as Minimum inhibitory concentration (MIC), Minimum bacteriocidal concentration (MBC) and Minimum fungicidal concentration (MFC) are the most basic antimicrobial susceptibility testing methods to determine the lowest concentration of any remedy which

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minimize (one or two colonies) or prevents (that gave no growth) of pathogenic organism (Atlas *et al.*, 1995; Chenoll *et al.*, 2006). This study was designed to isolate and identify *Lactobacillus spp.* from vagina and evaluate the effect of vaginal *Lactobacilli* on growth of urogenital pathogens as well as determining MIC, MBC and MFC against some pathogenic bacteria which isolated from vagina.

## Materials and Methods

*Lactobacillus spp.* were isolated from vaginal swab of 50 healthy women who attended to obstetric and gynecology clinic in Ibn-AL Baladi Hospital, Baghdad. Vaginal swabs were but in Manns-Regoz and Sharpe (MRS) broth (Hi media, Italy) and transported to Laboratory where inoculated into MRS agar with PH 5.2 and incubated at 37°C for 48 hr., then in 4.3 at in CO<sub>2</sub> candle jar for 48 hr. *Lactobacillus spp.* were identified according to colony morphology, gram stain biochemical tests and carbohydrate fermentation to identify species of *Lactobacillus* (Kandler and Weiss, 1986; Teuber, 1995; Holzapfel, 1997).

### Identified urogenital pathogens

Urogenital pathogens were isolated from female patients who attended to obstetric and gynecology department at Ibn-AL Baladi Hospital. Suffered from urogenital infection, pathogens were Identified depending on the basic methods to recognize these organisms according to (Baron and Fine, 1990; Jawetz *et al.*, 1998) furthermore antibiotic susceptibility test was done by disk diffusion method according to (Reller *et al.*, 2009) to determine resistance of selected strains to different antibiotics

### Evaluating antimicrobial activity of *Lactobacillus spp.* against urogenital pathogens

• **Agar plug diffusion method:** Antimicrobial activity of *Lactobacillus* strains supernatants against urogenital pathogens was tested by agar plug diffusion method on MRS agar according to (Pfaller *et al.*, 2004; CLSI, 2012) briefly, a cork borer 5mm was used to with draw disk of *Lactobacillus* strains growth after incubation and put on surface of Sabourauds dextrose agar that was inoculated (before) with 0.1 ml of yeast suspension and nutrient agar was inoculated with 0.1 ml of bacterial suspension. Then incubated at 37°C for 48 hr., the inhibition zone around the disk was estimated in millimeter.

• **Agar well diffusion method:** Liquid medium (MRS broth) was inoculated by 1% *Lactobacillus* strains and incubated 37°C for 48 hr., after incubation the culture was centrifuged at 6000 rpm for 15 min, filtrated through

millipore filter unit (0.22). According to well diffusion method that mentioned by (Magaldi *et al.*, 2004; Valgas *et al.*, 2007), nutrient agar plates were inoculated with 0.1 ml of pathogenic organisms by spreader, then 5 mm wells were made by cork borer, each well was filled with *Lactobacillus* supernatant and incubated at 37°C for 48 hr.

### Determining MIC, MBC and MFC of vaginal *Lactobacillus*

This test was done to determine the lowest concentration of *Lactobacillus* supernatant recovered from vagina which minimize (MIC) or prevent (MBC) and (MFC) the growth of pathogenic organisms isolated from female genital tract, the test was done according to (Jiménez and Roane, 2005; Elleuch *et al.*, 2010; Lertcanawani and Sawangnop, 2008), procedure briefly preparation different dilutions of *Lactobacillus* supernatant in sterile tubes containing normal saline to make final volume of 10 ml in each tubes at concentrations 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90%. Preparation pathogenic microorganisms suspensions which were compared with McFarland tube No. (0.5) which contain  $1.5 \times 10^8$  microorganisms. Preparation tubes containing 80 microliters of nutrient broth, added 100 microliters of pathogenic culture and 100 microliters of each dilution of *Lactobacillus* supernatant, incubated at 37°C for 48 hr., after that inoculation test plates by streaking Muller-Hinton agar plate surfaces by sterile cotton swabs and incubated at 37°C for 48 hr., the results observed and MIC detected by the lowest concentration of *Lactobacillus* supernatant that inhibit the growth of pathogenic microorganisms (on or two colonies), MBC or MFC the that gave no growth lowest concentration of pathogenic microorganisms.

## Results and Discussions

Suspected lactobacillus colonies were appeared pale, round, convex, soft, mucoid and surrounded by zones as result of dissolving calcium carbonate. Furthermore biochemical test conferred the diagnosis of lactobacillus isolates. Carbohydrate fermentation testes were done to identify *Lactobacillus spp.* which isolated from vagina. In this study vaginal *Lactobacillus* species were *L. acidophilus*, *L. crispatus*, *L. gasseri* and *L. iners*, A well as urogenital pathogens which were isolated from vagina were *Candida albicans*, *Gardenella vaginalis*, *Staphylococcus aureus* and *Escherichia coli* which were identified by routine diagnosis tests by gram stain and biochemical tests. Also all urogenital pathogens were resistant to Gentamicin, Streptomycin, Trimethoprim, Neomycin, Ampicillin, tetracycline, Erythromycin, Chloramphenicol and Cefalexin.

**Table 1:** Inhibition activities of vaginal *Lactobacillus* by agar plug diffusion method.

Lactobacillus strains	<i>Candida albicans</i>	<i>Gardenella vaginalis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
<i>L. crispatus</i>	15	16	18	17
<i>L. acidophilus</i>	16	17	18	20
<i>L. gasseri</i>	18	22	22	23
<i>L. iners</i>	17	18	19	22

**Table 2:** Inhibition activities of vaginal *Lactobacillus* by agar well diffusion method.

Lactobacillus strains	<i>Candida albicans</i>	<i>Gardenella vaginalis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
<i>L. crispatus</i>	16	17	20	18
<i>L. acidophilus</i>	17	19	19	19
<i>L. gasseri</i>	20	23	22	24
<i>L. iners</i>	19	19	21	23

The results of diagnosing urogenital pathogens which isolated from vagina showed that the most present organisms were *Candida albicans*, *Gardenella vaginalis*, *Staphylococcus aureus* and *Escherichia coli* and the results of investigation the antimicrobial activity of vaginal lactobacillus against these pathogens. Among four *Lactobacillus* species were observed to have bacteriocin activity *L. crispatus* had the highest effect on urogenital pathogens in both methods-Agar plug diffusion method and Agar well diffusion method-however all four strains showed good inhibition activity against urogenital pathogens as shown in table 1 and 2.

Current results indicated that *L. gasseri* have the highest inhibitory effect on indicator pathogens and that may due to *L. gasseri* production  $H_2O_2$ .

Present results indicate that *L. crispatus* have the highest inhibitory effect on indicator pathogens and that may due to the antimicrobial compounds such as bacteriocin like substances and biosurfactants regardless of  $H_2O_2$  production suggesting that they might produce some antimicrobial compounds in addition to  $H_2O_2$ , organic acid production and consequent pH reduction.

The results of MIC, MBC and MFC of vaginal *Lactobacillus* revealed that the *Lactobacillus* supernatant

**Table 3:** MIC, MBC and MFC of vaginal *Lactobacillus* against pathogenic isolates.

Pathogenic isolates	<i>Lactobacillus</i> supernatant concentrations									
	10%	20%	30%	40%	50%	60%	70%	80%	90%	
<i>G. vaginalis</i>	+	+	+	+	+	w	-	-	-	
<i>S. aureus</i>	+	+	+	+	+	w	-	-	-	
<i>E. coli</i>	+	+	+	+	+	w	-	-	-	
<i>C. albicans</i>	+	+	+	+	+	+	+	w	-	

Normal growth = +, Weak growth = W and No = No growth.

concentrations at 10%, 20%, 30%, 40% and 50% had no effect on growth of *G. vaginalis*, *S. aureus* and *E. coli* when clear growth was noticed while the concentration 60% led to minimize (MIC) growth these bacteria and concentration 70% inhibit (MBC) the growth completely, whereas sharp decrease in *C. albicans* growth (MIC) noticed in 80% concentration even as the concentration 90% and above inhibited all pathogenic isolates (MBC and MFC) as shown in table 3.

The result considered the concentration 60% was regarded as MIC against growth of *G. vaginalis*, *S. aureus* and *E. coli* was closely related with results of (Kubba, 2006) who noticed that the 60% concentration was MIC which minimized growth *S. aureus* and *Pseudomonas aeruginosa* and the value 70% regarded as MBC agree with the results who obtained by (AL-Yas, 2006) which found the same value *Lactobacillus* MBC against helicobacter pylori and (Lateef, 2006) against *Candida*. *Lactobacillus* are natural resistance factors against potential pathogenic microorganisms by producing autogenic regulation factors e.g. organic acids, hydrogen peroxide and bacteriocins.

It was clear that there is strong correlation between the present commensally microorganisms, particularly in vagina when *Lactobacillus* are absence urogenital infections well over growth (Mohamed and Jiyad, 2012; Mohamed and Thwani, 2009).

Vagina and its unique microflora form a finely balanced ecosystem, with the vaginal environment controlling the microbial types present and the microflora in turn control the vaginal environment (Mohamed and Thwani, 2009). This ecosystem is dynamic with changes in structure and composition being influenced by age, menstrual cycle, pregnancy, infections as well as a various habits and practices such as douching (Arshad *et al.*, 2018; Wagenlehner *et al.*, 2018). In recent years, the use of *Lactobacillus* as biotherapeutic agents had received wider attention and several studies provide evidence supporting the ability of *Lactobacillus* to prevent infections (Jung *et al.*, 2017). In current study, vaginal instillation of freeze-dried *Lactobacillus* suppositories was shown to result in 78% reduction in the incidence of recurrent UTI (Vodstrcil

*et al.*, 2015). Although many commercially available *Lactobacillus* products can be found in healthy food stores, their reliability is questionable and there is only little evidence proving their efficacy (Brookheart *et al.*, 2019).

Other drawbacks of the currently available products include poor product, viability and possible contamination with other organisms (Ng *et al.*, 2018). Therefore current research efforts are directed preparing safe and efforts are directed towards preparing safe and effective *Lactobacillus* preparations, this involves the involves the careful selection of strains with specific properties shown to be important in the interference of uropathogenic adhesion (Denkova *et al.*, 2017).

The criteria for selection of effective probiotic strains have been proposed and should include verification of safety, colonization ability in the vagina and ability to reduce the pathogen count through competitive exclusion of adherence and inhibition of pathogen growth (Pereira *et al.*, 2018).

Present results showed that all species have high antimicrobial activity against urogenital pathogens, but this activity varied greatly especially *L. crispatus* which seemed had a better effect than other species with promising inhibitory spectrum of antimicrobial activities against urogenital pathogens.

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