



## Antibiotic susceptibility and biofilm formation of *Lactobacilli* spp. isolated from healthy and infected women with bacterial vaginosis

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### Abstract

**Background:** The vaginal microbiome harbors diverse communities of microorganisms, known as vaginal flora which has an important impact on women's health as well as that of their newborns. Bacteria that colonize the vaginal microbiota of women play an important role in health and homeostasis that exist in a dynamic balance and establishing with each other and with the host. In healthy reproductive-aged women, the vaginal microbiome generally shows a predominance of *Lactobacillus* genus, and most women display the prevalence of one species among *L. crispatus*, *L. iners*, *L. jensenii* and *L. gasseri*. *Lactobacilli* promote the maintenance of the vaginal homeostasis and prevent the colonization and growth of adverse microorganisms, especially pathogens. **Methodology:** 150 high vaginal swabs were collected from 100 specimen from women suffering from vaginitis and 50 specimen from healthy women. Each specimen were cultured on UTI chromogenic agar under aerobic conditions and on MRS agar under anaerobic condition. Antibiotics susceptibility performed according to NCCLS (National Committee for Clinical Laboratory Standards). Biofilms formation were assayed by TCP. **Results:** Results revealed that a total 150 specimens, 90 were gave positive cultured with single bacterial growth on UTI chromogenic agar as following: *Escherichia coli* (42/90), *Staphylococcus aureus* (27/90), *Enterobacter* (13/90), *Klebsiella pneumoniae* (8/90), 30 specimens were gave mixed culture and 30 others specimens were no growth. Out of 150 specimens also cultured on MRS agar, 30 specimens were gave positive culture with pure bacterial isolates of *Lactobacillus* spp. 20 isolates of *Lactobacillus* (10 isolates from healthy women and 10 isolates from infected women with vaginitis) were selected to study the effect of antibiotics. It has been found that all *Lactobacillus* isolated from healthy women specimens resistant for chloramphenicol and amikacin. 50% resistant for ceftriaxone, 40% resistant for ciprofloxacin, gentamycin, and trimethoprim-sulfamethoxazole, 30% resistant for nitrofurantoin, 20% resistant for levofloxacin, and all *Lactobacillus* isolates sensitive to imipenem while all *Lactobacillus* isolates of women with vaginitis were sensitive to all antibiotics used in this study. Also 20 *Lactobacillus* isolates were selected to study the ability of them to produce biofilm formation. The *Lactobacillus* isolates from healthy women produce strong and moderate biofilm former 50%, while *Lactobacillus* isolates from vaginitis women produce (0%, 40%, 40%, 20%) strong, moderate, weak and non-biofilm former respectively. **Conclusion:** Most vaginal infections are treated by antibiotics but frequent recurrences and chronic infections are common due to the adverse effect on the indigenous *Lactobacilli*. Probiotics have the potential to optimize, maintain and restore the microflora of the vaginal ecosystem hence the alternative approach to reducing vaginal infections and promoting consumer health by using biotherapeutic with pre- and probiotic. The types of antibiotics used to treat vaginitis must not to kill the beneficial bacteria (*Lactobacilli*) that help in preservation of vaginal health and ecosystem as being one of probiotic bacteria.

**Keywords:** *Lactobacilli*, healthy, women, vaginitis

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### INTRODUCTION

The aim of this study is to compared among *Lactobacilli* isolated from healthy and vaginitis women in their antibiotics susceptibility and ability to produce biofilm formation. The vaginal microbiota microflora constitutes a complex micro-ecological environment composed of different microbiological species in variable quantities and relative proportions. The term vaginitis is the diagnosis given to women who present complaining

of abnormal vaginal discharge with vulval burning, irritation or itching. Inflammation of the vaginal mucosa, called vaginitis is one of the most frequent complaints in women attending gynecological clinics according for 10 million office visits each year. Aerobic vaginitis (AV) is

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caused by a displacement of the healthy vaginal *Lactobacillus* species with aerobic pathogens such as *Enterococcus faecalis*, *Escherchia coli* and *Staphylococcus aureus* that triggers a localized vaginal inflammatory immune response.(Sangeetha, et al. 2015). More than 250 species of bacteria have already been detected by genomic sequencing in a healthy vaginal environment and Lactobacilli are usually the prevalent microorganisms in women in reproductive age. (Martin, 2012; Mitiku, & Tadesse, 2018). The species most frequently found are *Lactobacillus crispatus*, *L. gasseri*, *L. jensenii*, *L. johnsoni*, *L. iners*, which protect the mucosa against the establishment of pathogenic microorganisms such as human immunodeficiency virus (HIV) and those associated with bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and gonorrhoea (Danielsson, et al. 2011). This protection is mainly due to the ability of lactobacilli to adhere to vaginal epithelial cells and inhibit the growth of pathogens, For this purpose, different mechanisms are employed, including immunodulation, production of antimicrobial substances, such as organic acids, hydrogen peroxide and bacteriocins, competition for nutrients and inhibition of pathogens adhesion to epithelial receptors by steric exclusion / displacement, biofilm production and /or co-aggregation (Petrova, et al. 2015).

## MATERIALS AND METHODS

### Isolation of Bacteria

During a period study ranged from November 2019 to February 2020, 150 high vaginal swabs from (100) specimens were collected from women suffering from vaginitis and admitted to Babylon Hospital of Maternity and Paediatrics in Babylon province. vaginal swabs were also taken from private clinics, and (50) specimens were collected from healthy women. The women age ranged from 20-50 years married and non-pregnant. All vaginal swabs were cultured on MRS broth and MRS agar under anaerobic conditions at 37°C for 24-48 hours.(Rogosa, & Sharpe, 1963). All vaginal swabs also inoculated onto brain heart infusion broth and then onto UTI chromogen agar and cultured under aerobic conditions for 24-48 hours at 37°C incubator at 37°C, then keep until used.

### Biofilm Formation Assay

The biofilm formation among *Lactobacillus* isolates were investigated according to tissue culture plate method (TCP) assay (also called semi quantitative microtiter plate test (biofilm assay) described by (Christensen, et al. 1985). Also, the results can be interpreted according to Stepanović, S., Vuković, D., Hola, V. (Bonaventura, et al.2007).

### Antibiotic Susceptibility Assay

Antimicrobial susceptibility testing of isolates was performed on Muller- Hinton agar plate (HiMedia,

**Table 1.** The Aerobic Bacterial Isolates on UTI Chromogenic Agar from Vaginosis and Healthy Women

Bacterial isolates	Patients women	Healthy women	Total
<i>E. coli</i>	35	7	42(28%)
<i>S. aureus</i>	26	1	27(18%)
<i>Enterobacter</i>	7	6	13(8.6)
<i>K. pneumoniae</i>	7	1	8(5.3)
Mix bacterial	15	15	30(20%)
No growth	10	20	30(20)
Total	100	50	150(100%)

Mumbai, India) by the Kirby-Bauer disk diffusion method following the National Committee for Laboratory Standards Interpretative procedure.(National committee for clinical and Laboratory Standards Institute. 2007.) The results were compared to those of *Staphylococcus aureus* ATCC29213 due to the absence of the *Lactobacillus* reference strains (Ocaña, et al. 2006). The antimicrobial agents tested were Ipemenum (IPM) Ceftriaxone (CRO) Trimethprime (TMP) Gentamycin (CN), Nitrofurantion (F), Levofloxacin (LEV), Amikacin (AK), Ciproflacin (CIP), Ceftrazidime (CTX), Standardized inoculum adjusted to 0.5 McFarland standard turbidity of each isolate was inoculated into Mueller – Hinton agar plates using a sterile cotton swab by streaking the swab over the entire sterile agar surface three times .Sterile forceps were used to place the appropriate antibiotic disks on the inoculated plate .The plates were incubated at 37°C overnight and then read the results.

## RESULTS AND DISCUSSION

During the study period range from November (2019) to February (2020), 150 high vaginal swabs (100 specimens were collected from women suffering from vaginitis and 50 specimens from healthy women and admitted to Babylon Hospital of Maternity and Paediatrics in Babylon province. Vaginal swabs were also taken married and non-pregnant. Out of 150 vaginal swabs culturing on UTI chromogenic agar, 120/150 specimens (90 from women with vaginitis and 30 from healthy women) 90/120 was gave positive culture with single bacterial species as following: *E. coli* (42/90), *S.aureus* (27/90), *Enterobacter* (13/90) , *K. pneumoniae* (8/90). 30/120 and 30/150 were gave mixed culture growth and no growth respectively (**Table 1**).

The negative bacterial cultures may be attributed to consumption of antibiotics by the patients, or the presence of another types of causative agents that might need a special techniques for their detection such as viruses, Chlamydia, and other agents or may be required to cultured the specimens under anaerobic conditions and to specific media to isolates a caustive agents of bacterial vaginitis and the mix culture may be attributed to there are to contamination occurred. Results of types and percentages of opportunistic bacterial isolates recovered from vaginitis patients in this study were correlated with the results obtained by

**Table 2.** The Anerobic Bacterial Isolates on MRSAgar from Vaginosis and Healthy Women

Bacterial isolates	Patients women	Healthy women	Total
<i>Lactobacillus</i>	10	20	30(20%)
Mix bacterial	55	25	80(53.3%)
No growth	35	5	40(26.6%)
Total	100	50	150(100%)

(Razzak, et al. 2011). revealed that a total of 110 bacterial isolates were obtained from women of vaginitis. The most of isolates were *Escherchia coli*, *Streptococcus agalactiae*, and *Klebsiella pneumonia* this results also agreement with that of (Mumtaz, et al. 2008) who found that 24% of the patients with aerobic vaginitis were infected with *S. aureus* gram positive cocci. The percentage of *Staphylococcus* species (*S. aureus*, *S. epidermitis*, *S. saprophytica*) in patients with vaginitis may reach to 62% (Forbes et al. 2007). The *K. pneumonia* in cases of vaginitis, may be attributed to taking of antibiotics by infected women ( especially B-lactam antibiotics) which inhibit the growth of other opportunistic pathogens, while *Klebsiella* isolates are considered the most common resistant bacteria to most antibiotics by producing extended spectrum B-lactamase.(Bedenic, et al. 2001). Hiller et al. (1993) studied the vaginal flora of some women and they found that organisms commonly isolated in women with normal smears were lactobacilli, coagulase negative staphylococci, *S. aureus*, diphtheroids, *Candida* and GBS. The isolation of *E. coli* from infected women with vaginitis were detected in this study and was also reported by Razzak, et al. (2011). The isolation of *Lactobacillus* from healthy and vaginitis women was also investigated. It was found that thirty isolates of *Lactobacillus* had been isolated anaerobically (20 isolates were from healthy women and 10 isolates were from infected women) **Table 2.**

Studies found that *Lactobacillus* colonized 71% of sexually active women (Antonio, et al.1999). In addition, the results showed that the presence of Lactobacilli together with other opportunistic pathogens may be due to several factors: effects of antibiotics, type of incubation (as some Lactobacilli species are unable to produce some defence factors under anaerobic incubation), and antagonism among Lactobacilli species to maintain dominance (Razzak, et al. 2011). The potential importance of Lactobacilli is to protect the vagina from disease, therefore, it has been used nowadays as probiotic (Andreu, 2004). In this study it was found from the result as shown in **Table 2** there were 80 specimens gave mixed cultures, this may be attributed to there are different types of lactobacilli in one specimen or related to contamination happened. 40 specimens were also giving no growth in this study, this may be related to consumption of antibiotics during treatment. Some antibiotics were used to show their effects on Lactobacilli species in vaginitis and healthy women. In this study 9 antibiotics were used to study the

**Table 3.** Percentage of Biofilm Formation among *Lactobacillus* spp. Isolates

Biofilm formation	Isolates NO.(%)	
	A	B
Non	2(20)	0(0)
Weak	4(40)	0(0)
Moderate	4(40)	5(50)
Strong	0(0)	5(50)

A: Women with vaginosis group , B: Healthy women

effects of antibiotics on Lactobacilli isolates and the isolates that gave positive pure cultures from women suffering from vaginitis and healthy women was investigated and results showed that all Lactobacilli (10 isolates) were isolated from healthy women resist to chloromphincol and amikiacin, 50% resist for ceftriaxone, 40% resist for ciproflaxcin ,gentamycin, and trimethoprim- sulfamethoxazole; 30% resist for nitrofuratoin; 20% resist for levofloxacin and 0% resist for imipenem. We suggest that the resistance to many antibiotics may be related to ability these isolates to produce biofilm former as shown in **Table 3.** These results were in agreement with the results obtained by (Testore, et al. 2002). who found that fifty isolates of Lactobacilli from 30 cervix specimens collected from 30 healthy women during colposcopy were tested for susceptibility to 41 antibiotics, it was found that aztreonam, fusidic acid, cefixime, kanamycin and pefloxacin showed less activity against Lactobacilli (72 – 86% of resistant isolates) all isolates were found to be susceptible to imipenem. Vancomycin showed higher antibacterial activity (22% of resistant isolates) than teicoplanin (54% resistance).

Antibiotic resistance in *Lactobacillus* does not constitute safety concer in itself, when mutations or intrinsic resistance mechanisms are responsible for the resistance phenotype. In fact, some probiotic strains with intrinsic antibiotic resistance could be useful for restoring its habitat microbiota after antibiotic treatment (Gueimonde, et al. 2013). Abdul – satter, et al (Abdul-satter, et al. 2011). also isolated 22 *Lactobacillus* isolates from vagina of healthy women and study the effects of antibiotics on *Lactobacillus* isolates and found all isolates resistant for Metronidazole that are the most commonly used for the treatment of bacterial vaginosis. These results suggest that selected strains could be used for restoration of therapy together with the antimicrobial bacterial vaginosis treatment and resistant to Ciprofloxacin, Nalidxic acid which helps in the selection of proposed probiotics should be taken by patients during antimicrobial therapy

In the present study there were found in addition antibiotics resistance also formation of biofilms in isolates of *Lactobacillus* that isolated from healthy vagina as shown in **Table 3.**

These results were similar to (Baksh, et al. 2018). showed 11 *Lactobacillus acidophilus* isolates from healthy women form biofilms with different levels (weak, moderate and strong) and inhibit the growth of

*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* isolates. The inhibition effect of biofilm formation supernatant of *Lactobacillus acidophilus* isolated from healthy women on pathogenic isolates of *E. coli*, *Acinetobacter*, *K. pneumoniae*, *Enterobacter aerogenes*, and *Staphylococcus aureus* was investigated by (Obaid, 2019). In this study, 10 isolates were isolated from 10 vaginal specimens were collected from vaginitis women to study the effect of antimicrobial agents on *Lactobacilli* isolates, it was found that all *Lactobacillus* isolates sensitive to all antibiotic used in this study and this result may be in relationship with results obtained from **Table 3** that isolates of *Lactobacillus* produce biofilms with different levels weakly, moderate and no produce biofilm former).

## CONCLUSION

Most vaginal infections are treated by antibiotics but frequent recurrences and chronic infections are common due to the adverse effect on the indigenous lactobacilli. Probiotics have the potential to optimize, maintain and restore the microflora of the vaginal ecosystem hence the alternative approach to reducing vaginal infections and promoting consumer health by using biotherapeutic with pre- and probiotic. The types of antibiotics used to treat vaginitis must not to kill the beneficial bacteria (lactobacilli) that help in preservation of vaginal health and ecosystem as being one of probiotic bacteria.

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