Isolation of Lactobacillus Spp. from First Trimester Pregnant and Determination their Antimicrobial Activity

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Abstract

Objective: Lactobacilli species that are more adapted to the vaginal environment of women may colonize and defend against harmful bacteria in the vaginal area. Methodology: 90 isolate of Lactobacillus spp. were isolated anaerobically from bacterial vaginosis and non- bacterial vaginosis pregnant women in first trimester. Confirmation diagnosis by VITEK-2, the antibacterial activity of bacteriocin extracted from Lactobacillus spp. is estimated using susceptibility tests. Results: Two isolation species of Lactobacillus from pregnant women in first trimester are confirmed, 5 antibiotics are used against 10 isolates of Lactobacillus, 100% resist for Cefixime ,80% resist for Cefepime ,60% resist for Nitrofuranation ,60% resist for Amoxicillin/lavulanic, 30% resist for Amoxicillin. Antibacterial activity of bacteriocin are positive against tow pathogenic bacteria. Conclusion: Lactobacillus spp. is a probiotic when establishing and maintaining a healthy vaginal balance.

Keywords: Lactobacillus, Bacterial vaginosis, Bacteriocin.

1. Introduction

Lactobacillus is a Gram-positive bacillus genus including over 180 species that require microaerophilic or strictly anaerobic environments. They colonize the lower section of the GIT, the vaginal area, and the oral, becoming part of the human microbiome. They're especially important in the female vaginal tract, where lactic acid production lowers pH and so protects it from bacterial and fungal diseases. Chemicals that are bactericidal and bacteriostatic, like bacteriocin and hydrogen peroxide (H2O2), are also produced by these bacteria, which are capable of eradicating rival species from their habitat [1, 2].

The beneficial bacteria in the vaginal ecosystem, such as Lactobacillus spp., is assumed to have evolved through mutualistic connection with its human host. It's critical to investigate the Lactobacillus spp. diversity of the vaginal flora in health and disease, as well as whether changes in individual vaginal Lactobacillus spp. can be linked to changes in vaginal infections. Bacterial vaginosis (BV) is a frequent vaginal illness caused by a microbiota imbalance [3-6].

The body of a pregnant woman undergoes several hormonal changes. Progesterone, prolactin, and estrogen levels grow as the pregnancy continues. The thyroid hormone thyroxine is also secreted in greater quantities. Placental hormone like placental gonadotropen, human placental lactogin, cortecotropen, placental tyreotropen, and steroid hormone also impact the mother [7]. Pregnancy affects the microbiome, as well as the immune and metabolic systems, increasing the risk of vaginal infection [8-10]. Lactobacillus spp. are probiotic bacteria that are non-pathogenic and produce antimicrobial chemicals. This is defined as "live microorganism that, when administered in suitable concentrations, provide a health benefit to the host" [11].

Lactobacillus spp. natural products as topical microbicides Lactobacillus spp. and other bacteria produce antimicrobial peptides (bacteriocins) as a defensive mechanism against competitor microbes in order to protect their ecological niche [12]. Their antibacterial effect is due mainly to permeabilization of the cell membranes, which result in the efflux of ions and ATR amino acids, reducing the trans- membrane potential () and pH gradient [13, 14].

In this present study, Lactobacillus spp. isolated from first trimester pregnant with bacterial vagenosis and non-bacterial vagenosis as control, conformation all isolates by Vitek-2, susceptibility test to Lactobacillus spp. isolates then extraction of bacteriocin from Lactobacillus spp. and determined their antibacterial activity.

2. Material and Method

Specimens Collection

90 High vaginal swab (HVS), they were collected from admitted patients to Imam Ali general hospital/ Hilla city and private clinics, HVS swab collected using sterile cotton swab without media. Samples were distributed as (50) sample from first trimester pregnant women suffering from bacterial vaginosis, and (40) from healthy first trimester pregnant women as control group.

Laboratory Diagnosis

Isolation and identification of bacterial isolates

All vaginal swabs were grown on MRS agar and MRS broth for 24-48 hours under anaerobic conditions at 37 °C. When a colony appears, it is identified depending on its morphology (size, colony shape, color, pigment nature, boundary, elevation, and texture) and then studied under a light microscope after being stained with Gram's stain. 40 percent glycerol storage.

VITEK- 2 assay

Isolated Lactobacillus spp. were identified by using VITEK-2 ANC card for further identification (according to

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manufacture instructions).

Antibiotics susceptibility test

"The clinical laboratory standard Institute (CLSI)" recommendations 2021 were used to evaluate antibiotic susceptibility using the "Kirby-Bauer disk diffusion technique". In this study 5 antibiotics Cefixime (CFM-5 µg), Cefepime (FEP-10 µg), Nitrofuranation (F-100 µg), Amoxicillin\Clavulanic acid (AMC-30 µg) and Amoxicillin (AX-25 µg) against 10 Lactobacillus spp. isolates to evaluate the susceptibility of Lactobacillus spp. isolates (chosen at random from the total isolate).

Antibacterial activity of bacteriocin

The activity of antimicrobial of Lactobacillus spp. bacteriocin against various pathogenic bacteria was determined using the well diffusion method [15]. In nutritional broth, pathogenic organisms such as S. aureus and E. coli were implanted. The bacterial concentration was adjusted to 0.5 McFarland's standards and sub-cultured on Muller Hinton agar after an overnight incubation at 37oC. Following that, 100 mL of CFCS was poured into 8 mm diameter wells punched in Muller Hinton agar plate, which were then cultured for 18-24 hours at 37 ° C. The antimicrobial properties was then measured as a growth-inhibitory zone surrounding the well.

3. Results and Discussion

Confirmative Diagnosis of Lactobacillus spp. (Colony Morphology and Microscopic Examination)

90 isolate of Lactobacillus spp. were isolated anaerobically from bacterial vaginosis and non- bacterial vaginosis pregnant women in first trimaster. These bacteria were previously diagnosed microscobicaly. Lactobacillus spp. were identified depending on the morphological characteristics like (shape, colony size, color, transparency, edge, natural of pigment, elevation), it looks like on the agar. Table (1)

Table (1):Colony morphology and microscopic examination of Lactobacillus spp.

Tests	Result
Growth on selective media (MRS)	+ve
Colony morphology	Circular, large, smooth, glistering, White, colorless and creamish
Gram stain reaction	G+v, baclli

Table (1), show the colony and bacterial cell morphology was Gram-stain was used to examine the shape, arrangement, and type of reaction with positive Gram-stain.

Distribution of VITEK-2 results according to study groups

The system VITEK -2 was used to confirm the bacterial results of previous identification between bacterial vaginosis and non-bacterial vaginosis pregnant, the result probability between (98-99%), are show in Table (2).

Table (2):Disterbution of diagnostic *Lactobacillus* spp. isolated by Vitek-2 compact system by BV and non-BV pregnant

Vitek-2	Groups of study				P-value
	BV		Non-BV		
	F	%	F	%	
L. casei	3	6.0	11	27.5	0.001 H.S
L. gasseri	4	8.0	19	47.5	
No growth	43	86.0	10	25.0	
Total	50	100.0	40	100.0	
F= frequency					

Two isolation species of Lactobacillus from pregnant women in first trimester, the most frequent species populating the vagina in the first trimester among the patients evaluated were: L. casei 6.0% of BV and 27.5% of non-BV, L. gasseri 8.0% of BV and 47.5% of non-BV. Our study corresponds to this study [16] , Lactobacillus casei has been found to dominate among Lactobacillus. spp in healthful women vagina samples.

Table (2) show that a highly significant differences between BV and non-BV groups so that more than three quarters 43 (86%) of BV group were no growth, while 19 (47.5%) of non-BV group were L. gasseri, while 11 (27.5%) of non-BV group were L. casei.

The lactobacilli communities observed in Mexican vaginal groups included L. gasseri, L. rhamnosus, L. fermentum, L. crispatus, L. jensenii, and L. brevis. Since the dominant Lactobacillus species varies by race and area, be able to determine lactobacilli at the species level must aid our understanding of their functions. To overcome the difficulty of isolating these bacteria and avoid misidentification in future studies, culture-independent methods must be used [17].

Antibacterial activity of Bacteriocin isolated from Lactobacillus spp.

Antibacterial activity of bacteriocin against various pathogenic bacteria were (E. coli and Staphylococcus aureus) obtain on inhibition zone in different diameters, as shown in the table (3) and Figure (1)

Table 3:Antibacterial activity of bactriocin from Lactobacillus spp.

Pathogenic bacteria	L. casei	L. gasseri
E. coli	14mm	13mm
S. aureus	19mm	22mm

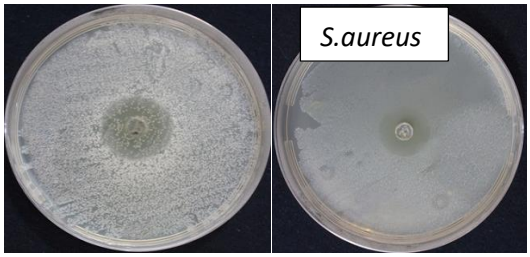
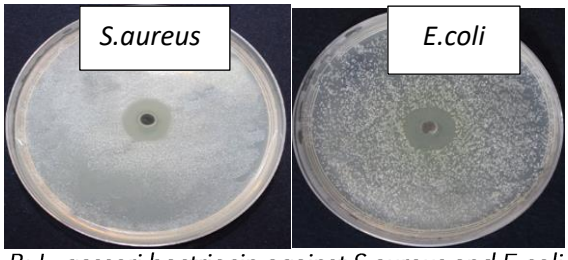


Figure (1): A: L. casei bactriocin against S.aureus and E.coli



B: L. gasseri bactriocin against S.aureus and E.coli

The mechanisms by which Lactobacillus spp. inhibit the colonization of other bacterial species in the vaginal cavity define their effectiveness in treating vaginal

dysbacteriosis with or without other pathogenetic or aetiological therapies [18, 19].

Lactobacillus spp. By producing defensive factors, they play a crucial role in sustaining vaginal health, and certain of these defense factors have an inhibitory effect on opportunistic infections (S. aureus and E. coli), as evidenced by Kato et al. [26] who observed that Lactobacillus spp. populate the typical vagina of reproductive-age women, producing hydrogen peroxide, bacteriocins, and lactic acid. These compounds can reduce the vaginal pH, making the environment more unfriendly for bacteria other than Lactobacilli. When the quantity of Lactobacillus spp. decreases, the pH rises, favoring an overgrowth of anaerobic and facultative bacteria, which can lead to virginities/vaginosis.

Gardnerella vaginalis proliferation was suppressed by vaginal and probiotic Lactobacillus isolates in a strain-specific manner. This suggests that Gardnerella vaginalis genotype may play a role in Lactobacillus-mediated inhibitory susceptibility. Metronidazole-resistant G. vaginalis strains were found to be less responsive to Lactobacillus spp. suppression in previous experiments [20].

Antibiotics susceptibility of Lactobacillus spp.

In healthy women and women with vaginosis, antibiotic have been given to see how they affected Lactobacillus species. Five antimicrobial agents [Cefixime (CFM-5), Nitrofuranation (F-100), Cefepime (FEP-10), AmoxicillinClavulanic acid (AMC-30), and Amoxillin (AX-25)] are being used in this research to determine the effects of medications on Lactobacillus isolated strains that gave positive pure culture from women suffering from bacterial vaginosis and healthy women (figure) (1). The findings revealed that all Lactobacillus (10 isolates) extracted from vaginosis-affected women were sensitive to all antibiotics tested, whereas all Lactobacillus (10 isolates) obtained from healthy women were both sensitive and resistant to all antibiotics tested. As in Fig.(2):

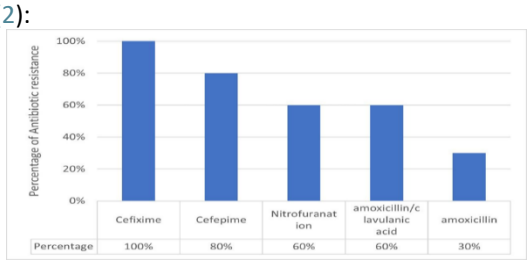


Figure (2): Percentage of Antibiotic Resistant among Lactobacillus spp. Isolates in healthy women

In this study all Lactobacillus (10 isolates), 100% resist for Cefixime ,80% resist for Cefepime ,60% resist for Nitrofuranation ,60% resist for Amoxicillin/lavulanic, 30% resist for Amoxicillin.

The high rates of recurrence of BV after treatment with currently approved antibiotics has always been a source of contention. These findings could be explained by one of two ways. For starters, BV-associated bacterium and multi - species BV biofilms might play a part in the disease's likely pathogenic pathway [21] . Resistance to antibiotics in Lactobacillus is not a cause for worry when the resistance phenotype is caused by mutation or internal resistance mechanism. Indeed, certain probiotic strain with built-in resistance to antibiotics may be useful for re-establishing the microbiota of an animal's habitat after antimicrobial treatment [22].

Abdul-sattar et al. [23] extracted 22 Lactobacillus isolates from normal women's vagina and investigated the effect of medications on Lactobacillus isolates, discovering that all isolates exhibited resistance to Metronidazole, the most generally used antibiotics for bacteria vaginosis therapy. These results show that some isolates might be utilised to restore treatment in combination with antibiotic bacterial vaginosis therapy and resistant to Ciprofloxacin, and also Nalidxic acid, which assists in the choosing of suggested probiotics that patient must take throughout antibiotic treatment [23].

Razzak et al. [24] Antimicrobial therapy's impact on Lactobacillus was investigated, and it was determined that the antibiotics had an obvious impact on Lactobacillus. Antibiotic may kill both helpful and harmful bacteria in the body, so they can also induce a microflora imbalance with serious effects, such as the removal of microflora from vaginal epithelial cells, enabling bacteria to adhere. As a consequence, patients must use medications to treat vaginitis with extreme caution to prevent killing lactobacilli [24]. \s. Whereas antibacterial agents are typically efficient in eliminating the illness, there is a high frequency of relapse, which impairs the patients' quality of life, and also many women grow irritated even by cycle of reporting antibacterial drugs, whose efficiency is decreasing due to the rising emergence of resistant bacteria. Furthermore, the use of antimicrobial agents may disturb the body's balance by killing off good bacteria, that can disturb the patient's quality of life [25].

4. Conclusion

In the present study, we have demonstrated that the Lactobacillus species microbiome in the vaginal microbiota of pregnant women changes over time between BV and non-BV in the three trimesters pregnant. Several Lactobacillus isolates exhibit antibacterial activity against pathogenic bacteria. We suggest that this effect may have potential applications through the use of Lactobacillus isolates as probiotics to prevent infection has a strong justification and a long track record of safety, particularly in the prevention of BV. It is vital that strains be defined and clinically tested using the preferred administration mechanism (oral, vaginal, dried powder, or in suspension). Women have the advantage of being able to self-administer probiotics. Many more research are needed to enhance the defensive properties of the vaginal microbiota, although probiotic intervention has the potential to improve the health of many women.

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