Antimicrobial activity of vaginal Lactobacillus against urogenital pathogens

Suhad Faisal Hatem Al-Mugdadi[1*], Ishraq Hasan Elewi, Zainab Farooq Shafeeq Al-Ryyis, Hala Muaed, Mays Ali

Abstract

Lactobacillus is one of the most important genera of Lactic Acid Bacteria (LAB). Lactobacilli present in a healthy vagina are part of normal bacterial microbiota and protect the host from urogenital infections by maintaining a low pH. Important features of probiotic lactobacilli are to achieve antagonistic activity against bacterial pathogens because of their capacity to produce lactic acid and other organic acids, H2O2 and bacteriocin. This study aimed to studying the effect of Lactobacillus spp. isolated from healthy vagina as aprobiotic against many clinical bacterial isolates from urogenital tract infection. Susceptibility to Five antibiotics, such as amikacin (AK), Tobramycin (TOB), Pipracillin (PRL), Nalidixic acid (NA) and Gentamycin(GN) was achived on all pathogenic bacteria isolated from urine and vagina. Two-fold dilutions of Lactobacillus sp. were prepared (1 × 108, 1 × 104 and 1 × 102) CFU/ml to determine antibacterial activity against urogenital bacteria isolates using agar well diffusion method. N. gonorrhoeae from vagaina was affected by all these dilutions of Lactobacillus sp. with inhibition zones 10, 8 and 8 mm respectively. While inhibition zones of S. aureus and Candida albiccans from vagaina were 12mm in dilution (1 × 104) and 6 mm in dilution (108) respectively. In addition, there was no biological activity of Lactobacillus sp. against bacteria isolated from urine. The effectiveness of natural lactobacilli from vagina consider as good therapeutics against bacterial illness of humans. We need future study to purification the active component like bacteriocin or other organic acids from these bacteria.

Keywords: Antibiotics; lactobacilli,vagina; Probiotic; Agar well diffusion method

* Correspondence author: suhadhatim@uomustansiriyah.edu.iq
Suhad Faisal Hatem Al-Mugdadi[1], orcid.org/0000-0002-7164-7093
Ishraq Hasan Elewi, orcid.org/0000-0002-7918-7853
Zainab Farooq Shafeeq Al-Ryyis, orcid.org/0000-0001-6514-7925
[1]University of Al-Mustansiriyah/ College of Pharmacy/Department of Clinical Laboratories sciences
Received 12 August 2017, Accepted 30 November 2017, Available online 27 December 2017

Introduction

Lactobacillus is one of the most important genera of Lactic Acid Bacteria [1]. Lactic acid bacteria (LAB) are Gram positive, non-spore forming catalase negative cocci or fermentative lactobacilli which produce lactic acid from fermentation of carbohydrates [2,3], facultative anaerobic, rod shape and non-spore forming bacteria [4]. The Genus Lactobacillus currently includes more than 170 different species [5] and only a part of them are representatives of protective vaginal microbiota [6]. Also, lactobacilli can participate in the protection of the vaginal epithelium by the means of several other barrier mechanism Lactobacillus could be used asprophylactic and therapeutic agents [7]. Lactobacilli present in a healthy vagina are part of normal bacterial microbiota and protect the host from urogenital infections by maintaining a low pH (<4.5), by producing bacteriostatic and bactericidal substances and through competitive exclusion [8,9]. Are excellent antibacterial agents (majore part of the vagainal micro biota [10,11]. Also, lactobacilli can participate in the protection of the vaginal epithelium by the means of a number of other barrier mechanisms Lactobacillus could be used asprophylactic and therapeutic agents [7]. One of the important features of probiotic lactobacilli is to achieve antagonistic activity against bacterial pathogens because of their capacity to produce lactic acid and other organic acids that lower the pH in the human intestine, and to produce H2O2 and bacteriocin, thereby establishing a hostile environment for the growth and survival of various human pathogenic bacteria [12,13]. Studied the effect L.acidophilus strains in combination with antibiotics, and found that the strains improved the antibacterial efficacy of the test antibiotics against E. coli, Salmonella typhimurium, S. aureus and Shigella flexeneri. However, no report has been made on the combined antibacterial activity of two different lactobacilli strains, at least in this part of the globe, against human pathogenic bacteria Probiotics are defined as live microorganisms which when administered in adequate quantity confer health benefits to the host and lactobacilli of human origin are potential probiotics against urogenital tract infections [14, 15]. Many isolated Lactobacilli were examined against multi-drug-resistant uropathogens, including E. coli, C. albicans, E. fecalis, P. aeruginosa and K. pneumoniae and showed a good resultes as antimicrobial agent [16]. Bacterial vaginosis and vulvovaginal candidiasis are the most common vaginal infections worldwide [17]. The first one is responsible for about 50% of all the cases and significantly associated with reduce level in lactobacilli population and increase in anaerobic and facultative aerobic pathogens [18, 19]. The second one affects about 75% of women with usual complaints like pruritus and vaginal discharge [20]. The majority of vulvovaginal candidiasis cases (90%) caused by Candida albicans [21, 22]. Among the Eleven vaginal lactobacilli isolates, L agilis, L. jensenii, L. johnsonii, and L. ruminus acted as potential probiotic species including the ability to co-aggregate with Candida species, adhere to epithelial mucosa, to auto-aggregate, and to create both H_2O_2 and lactic acid. Although H_2O_2 production by all strains, the highest levels were noticed for L. acidophilus, L. gasseri ,L. crispatus, L. vaginalis and L. johnsonii [23]. This study aimed to studying the effect of Lactobacillus spp. isolated from healthy vagina as a probiotic against many clinical

bacterial isolates from urogenital tract infection which are multidrug resistance.

Materials and Methods

Isolation and characterization of lactobacillus spp.

The lactic acid bacteria were isolated from healthy vaginal swabs on selective media. This isolate were cultured and stored in De Man Rogosa and Sharpe (MRS) media (Hi-Media, India) on agar plate or broth incubated anaerobic (5-10%) in candle jar for a period of 18-36h h at 37 °C [24]. From MRS agar plates, the diagnosis of lactobacilli sp. depended on morphology (gram positive rod-shaped bacteria), culture, non-motile, non-spore forming, negative oxidase and catalase test.

Clinical isolates

All clinical isolates used in this study were collected from patients suffered from urogenital clinical sign and symptoms and attended Bagdad teaching hospital-Iraq. All samples plated on agar media including Blood, Macconkey and Chocolate agar which were prepared according to the instructions of the Hi- media –India company. at 37°C for 18-24h. The most pathogenic bacteria were diagnosed by microscope examination, culture and some biochemical tests. Eight pathogens submitted in this study included: Four kinds of pathogens (K. pneumonia, E. coli, M. morganii and S. aureus) isolated from urine samples, also another four samples (N. gonorrhoeae, S. aureus, Proteus spp. and Candida albiccans) isolated from vagina.

Susceptibility pattern of clinical isolates

Susceptibility to 5 antibiotics, such as amikacin (AK), Tobramycin (TOB), Pipracillin (PRL), Nalidixic acid (NA) and Gentamycin (GN) (Hi-Media,India) were tested against urogenital pathogens by using disc diffusion method [25]. Single colonies of the test bacteria strains were inoculated into Muller Hinton agar and incubated at 37°C for 18-24 h. by cotton swab which was used to spread bacteria on the surface, and the antibiotic discs were placed on the surface of the agar plates. After incubation for 24 h at 37°C, the zone diameter of inhibition (ZDI) obtained around the antibiotic disc were recorded.

Fresh Lactobacillus spp.dilutions

Two-fold dilutions were prepared from lactobacillus sp. include ($10^{8,}$ 10^4 and 10^2) CFU/ml adjusting to McFarland to determine the effect all these dilutions against the pathogenic bacteria.100 ml of inoculums of each type of pathogenic bacteria was spread on muller- Hinton agar plate and blood agar. Then let the plate to dry then, six mm wells were made in agar plates. 100µl volume of each dilution ($10^{8,}$ 10^4 and 10^2) submitted in wells into media. All plates were incubated at (37°C for 18-36h.). Antimicrobial activity determined by clear inhibition zone surrounding the wells, results consider positive if the inhibition zone more than 6mm. completely inhibited the growth for 24h was recorded.

Results

Table (1) showed multidrug resistant bacteria againt five antibiotic types including disc of Amikacin (AK), Tobramycin (TOB), Pipracillin (PRL), Nalidixic acid (NA) and Gentamycin (GN). S. aureus isolated from urine and vagina in addition to N. gonorrhoeae from vagina were resisted to all five antibiotic types. K. pneumonia was sensitive to Amikacin and

Gentamycin and resist to others. Proteus spp. sensitive to Amikacin and Nalidixic acid and resist to others. Two-fold dilutions of natural lactobacillus sp. Isolate from healthy vagina were prepared as following ($1 \times 10^{8,1} \times 10^{4}$ and 1×10^{2}) N. gonorrhoeae from vagina was affected by all these dilutions with inhibition zones 10, 8 and 8 mm respectively. While inhibition zones of S. aureus and Candida albiccans from vagina were 12 in dilution (1×10^{4}) and 6 mm in dilution (1×10^{8}) respectively. Bacteria isolated from urine (K. pneumonia, E. coli, M. morganii and S. aureus) were resistant to all dilutions of lactobacillus sp., Table (2) and Figure (1).

Table 1

Sensitivity pattern of some antibiotic disk against many clinical bacterial isolates

Antibiotic disk / Bacterial isolates	AK	GN	PRL	NA	TOB
K. pneumonia (urine)	S	S	R	R	R
E. coli (urine)	S	R	R	S	R
M. morganii(urine)	S	R	R	R	R
S. aureus(urine)	R	R	R	R	R
Proteus spp.(vaginal)	R	R	R	S	R
S. aureus (vagina)	S	R	R	R	R
N. gonorrhoeae (vagaina)	R	R	R	R	R
Candida albiccans(vagaina)	-	-	-	-	-

Table 2

In vitro activity of newly isolated vaginal lactobacillus sp. dilutions against many pathogens

Lactobacillus sp. dilutions / Bacterial isolates	Diameter of inhibition zone (mm)		
	1×10^{8}	1×10^{4}	1×10^{2}
K. pneumonia (urine)	----------	----------	----------
E.coli (urine)	----------	----------	----------
M. morganii (urine)	----------	----------	----------
S. aureus (urine)	----------	----------	----------
Proteus spp. (vaginal)	----------	----------	----------
S. aureus (vagina)	----------	12mm	----------
N. gonorrhoeae (vagaina)	10mm	8mm	8mm
Candida albiccans (vagaina)	6mm	----------	----------

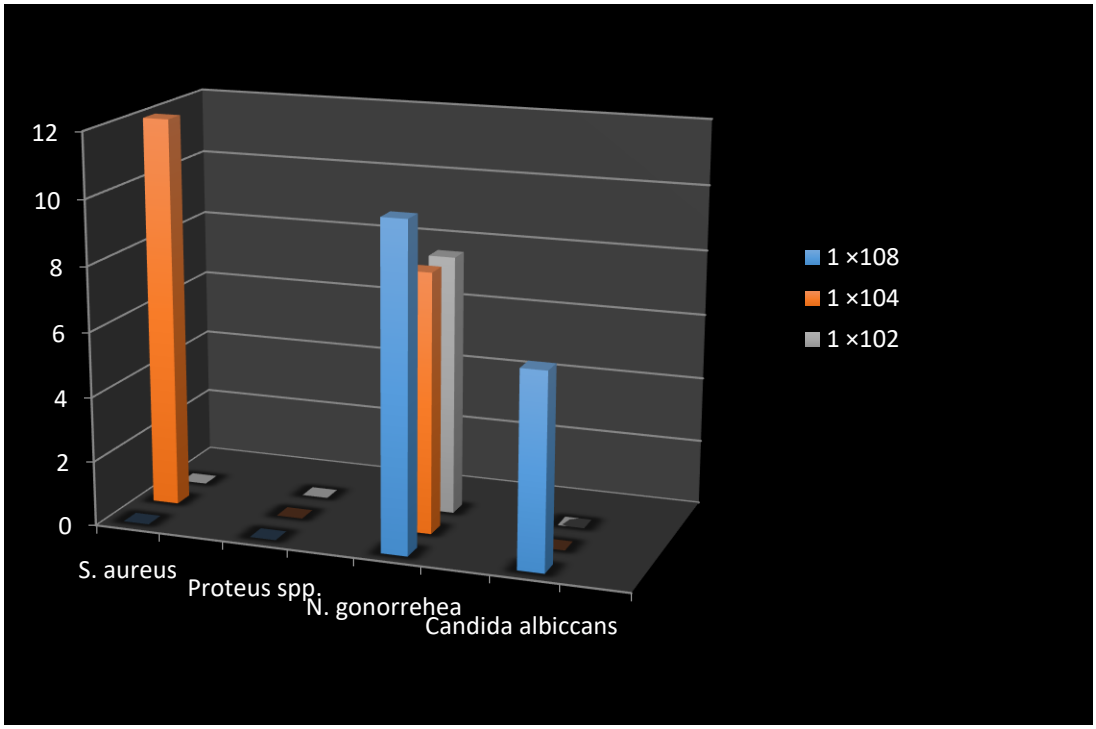

Figure 1

lactobacillus sp. dilutions activity against four kinds pathogens isolated from vagina

Discussion

This increasing in the emergence of drug-resistant pathogenic bacteria led to search the non-antibiotic treatment regimens, including the probiotic lactobacilli, as new therapies to fight the bacterial antibiotic resistance and to treat the disease [26-27]. The result in Table (1) showed resistant of bacteria to word Five antibiotics namely mikacin; Pipracillin; Gentamycin; Tobramycin and Nalidixic acid. Antibiotic resistant infections can occur anywhere in the community but also there has been an increase of infections that occur in the hospitals and other medical facilities. Resistance in bacteria is a large issue because as bacteria evolves and forms ways to make the antibiotics ineffective and harmful and fatal bacteria can thrive in multiple environments [28]. [29]. Cited that Strains of E. coli that are resistant to single or multiple classes of antibiotics,also pneumococcal resistance has increased to Beta-lactams,Macrolides and Lincosamides while M. Morganii& Proteus spp. isolates resistant to third-generation cephalosporins, imipenem, amikacin, and ticarcillin. As we know that bacteria resist to multi drug when it carries several resistance genes. It should be noted that a relatively high biological activity of half dilusions of Lactobacillus sp.

isolate isolated from healthy vagina (1×10^8 ,1 $\times 10^4$, 1 $\times 10^2$) against S. aureus, N. gonorrhoeae and Candida albiccans isolates from vagaina by mussering the inhibitory effect zone (mm) as shown in table(2),this result lined with [30,23]. The Lactobacillus species are usually selected as the probiotic microorganisms because of and bile, inhibitory effect against pathogenic microbes, and innate resistance to antibiotics. The production of lactic acid and other organic acids, hydrogen peroxide (H2O2) and bacteriocins help to keep the vaginal pH below 4.5 and creates a hostile ecosystem for the growth and survival of pathogenic microorganisms [23,31]. However, it did not inhibit other bacteria isolate in current study may be because the inhibitory activity of this bacteria (antagonistic effect) occurred between pH 3and pH 5 which it not adjusted in vitro. [32] Reported that several antifungal compounds, such as cyclic dipeptides, pyroglutamic acid and lactones were formed by Lactobacillus isolates and played an important role against Candida spp. in addition to ability of probiotic strains to adhere to epithelial mucosa, to auto-aggregate, co-aggregate with Candida species, and to produce both H2O2 and lactic acid. All the lactic acid bacteria screened for bacteriocin production, Lactobacillus bulgaricus, L. lactis, L. acidophilus, Lactococcuslactis, Streptococcus thermophilus, S. cremoris, Pediococcus halophilus and P. cerevisiae produced bacteriocin activity among 4800 and 6000 Au/ml against Staphylococcus, Salmonella, Shigella, Bacillus and Pseudomonas species [33]. The susceptibilities of the examined Gram-positive (S. aureus, S. xylosus and S. uberis) and Gram-negative bacteria (E. coli and Y.enterocolitica) to growth inhibition by the bacteriocin of Lactobacillus species were recorded [34], bacteriocin of L. fermentum (10 kDa) had no effect against S.aureus and Yersinia enterocolitica. Recently Heredia-Castro et. al., [35] recorded that Lactobacillus species from cheese were shown to produce bacteriocin-like substances active against S. aureus, L.innocua, E. coli and S. Typhimurium by using the disk diffusion. Another study showed that bacteriocins of vaginal lactobacilli were steady at pH 4.5 - 7 but sensitive to pH 9 [36]. May this stability lead to make lactobacilli more effective on vaginal pathogens. In conclusion: The effectiveness of natural lactobacilli from vagina consider as good therapeutics against bacterial illness of humans. We need future study to purification the active component like bacteriocin or other organic acids from these bacteria.

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