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Comparative study between effect of some antibiotic agents and probiotic lactobacilli species on the selected human urogenital pathogens

Jassim Hussein Mukharmash

Wasit University, College of Medicine, Department of Medical Microbiology.

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ABSTRACT

Probiotics are live microorganisms which are mainly strains of Lactobacillus species (spp.), and others. These microorganisms offer a health benefit for the host when administered in adequate amounts. Urinary tract infections (UTIs) samples were obtained from 42 patients who had been admitted to hospitals in Al-Karameh hospital during July 2016. These samples were inoculated onto enriched and differential culture media. Susceptibility of isolated pathogens to standard isolates lactobacilli spp. (10^7 CFU / ml) and twelve commonly used UT antibiotics was tested using standard susceptibility testing. Thirty one variance bacterial species were isolated. Escherichia coli (E coli) 9 (92.03%) were the most commonly isolated microorganisms, followed by Proteus mirabilis (P mirabilis) 6 (19.35%), Staphylococcus aureus (S aureus) 5 (16.13%), Klebsiella pneumonia (K pneumonia) 5 (16.13%), Pseudomonas aeruginosa (P aeruginosa) 4 (12.9%), and Staphylococcus epidermidis (S epidermidis) 2 (6.45%). In susceptibility testing, Lactobacillus spp. had coverage against 31 (100%). The coverage of the remaining twelve antibacterial agents used was different in their activity, which ranged between 50-100%. The results of the current study concluded that probiotics had a high effectiveness to inhibit the growth (in vitro) of all pathogens compared with antibiotics. This indicated the therapeutic efficacy of probiotics.

1. Introduction

Urinary tract infections (UTIs) are the most predominant bacterial infectious diseases with large financial expenses on community, and seen in clinical specimens. Human UTIs comprises disease entities, such as acute pyelonephritis with renal parenchymal involvement, cystitis limited to the urinary bladder, and asymptomatic bacteruria [1]. Selvamohan and Sujitha [2] clarified that UTIs are frequently accompanied with urologic disorders, and can cause final stage of renal failure or hypertension if continued. Enterobacteriaceae genera, which are normal inhabitants of gastrointestinal tract (GIT), account for the wide majority of these uncomplicated infections [3-4]. Appropriate hygiene and cleanliness of the genital area are therefore recommended for prevention of UTIs. The most of cases are caused by a limited number of bacterial genera, such as *E. coli* and *P. mirabilis* strains, which are responsible for more than 80% of the UTIs [5]. On the other hand, studies have shown a correlation between a loss and disruption of the normal genital microflora, in particular probiotic lactobacilli spp., and an increased incidence of urogenital infections [6]. Probiotics are defined as live microorganisms, principally bacteria, which are safe for human consumption [7]. Preclinical and clinical reports have focused on lactobacilli strains, their possible prophylactic effects against experimental *E. coli* infections, and the use of these strains for the prevention of human urogenital infections [8-9]. Naidu *et al*. [10] mentioned that when ingested in sufficient quantities, probiotics have beneficial effects for human health, beyond basic nutrition. These effects may result from suppression of pathogens and stimulation of probiotic growth that contributes to the nutrition and health of the GIT. *Lactobacillus* and various *Bifidobacterium* spp. consider the majority of probiotic bacteria. These probiotic bacteria are the most dominant bacteria in the GIT of humans that can inhibit the growth of different pathogenic microorganisms via production of antimicrobial substances e.g. organic acids (lactic and acetic acids), bacteriocins and others [11]. Chang *et al*. [12] reported that there are numerous strains of probiotic lactic acid bacteria (LAB), which reported to display stimulatory properties on cells of the innate immune system *in vitro*. These include natural (NK) and macrophages cells that induce adjuvant activity at the mucosal surface and improve phagocytosis by increasing the proportion of NK and lymphocytes cells. Moreover, probiotic LAB DNA can suppress systemic inflammatory responses to pathogenic bacterial DNA [13]. Madsen [14], Holly *et al*. [15] demonstrated that the explanation of therapeutic efficacy of probiotic bacteria may be clear through its ability to modulate epithelial barrier function, with possible interaction with toll-like receptor 2 (TLR-2). Through several researches, it is believed that LAB could protect sites of bacterial invasion from its colonization of pathogenic agents by preventing the attachments of these pathogens to sites. In addition, LAB produces several substances, which inhibit their multiplications by competing with other microorganisms for nutritional requirements. This might inhibit the multiplication of these causative agents by excreting substances, especially hydrogen peroxide (H_2O_2), organic acids, bacteriocins and others [16-18].

The aims of the current study to identify and diagnose the pathogens of bacterial species isolated from UT, and also

Tele: 009647725770775
E-mail address: jhmn2013@gmail.com

to estimate the therapeutic activity of lactobacilli spp. strains against pathogens. The activity of these strains was also compared to other antibacterial agents (antibiotics) activity, which are used medically in the treatment of UTIs.

2. Materials and Methods

2.1. Patients, different bacterial isolates and culture conditions

A total of thirty one UT swabs were collected and analyzed from patients who were clinically suspected of UTIs through summer 2016. The samples were collected at one location in the city of Al-Kut, Iraq, the laboratory of clinical microbiology of the Al-Karameh hospital. The swabs were collected from; male and female patients with mean age of 3-67 years. UT swabs were collected aseptically and transported immediately in sterile test tubes containing brain-heart infusion broth (BHIB) to hospital laboratory. The samples were examined and diagnosed by standard culture methods depending on colony morphology of bacterial isolates, microscopic Gram stain investigation and other parameters and tests, such as biochemical and analytical profile index (API) tests. All isolates were cultured on the following media; blood agar, BHIB, MacConkey agar, Manitol Salt agar (MSA), Mueller-Hinton agar (MHA), and tryptic soya agar (TSA) media (OXOID, Hampshire, UK), which incubated aerobically and anaerobically for 18-24 hours at 37˚C. The pathogens harvested through centrifugation at 3000 rpm for 10 min. and then washed three times with sterile phosphate buffer saline (PBS). Stander strain of lactobacilli; *L. fermentum* ATCC (American Type Culture Collection) 14931 and *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842 (*Lactobacillus bulgaricus*) were cultured in de Man Rogosa and Sharpe (MRS) broth and/or agar (OXOID, Hampshire, UK). Inhibition zones were recorded on Mueller-Hinton agar (OXOID, Hampshire, UK). Each probiotic lactobacilli strains were cultured and incubated aerobically or anaerobically in MRSB (10^7 CFU / ml) with a gas generating kit (5-10% CO_2 of atmosphere) at 37°C for 18-24 h.

2.2. Antibacterial activity

Antibacterial activity of lactobacilli spp. was tested against a variety of strains that became isolated from UTIs by using Agar spot method. This method was a modification of that described by Schillinger and Lücke [19]. Lactobacilli spp. suspension was performed for use in susceptibility testing with 1% concentration from liquid culture of bacteria that contain 10^7 CFU / ml [19]. The antibacterial agents (from Bioanalyse-Turkey) were Amikacin (AK) 30µg, Ampicillin (AM) 10µg, Cefotaxim (CTX) 30µg, Cefotaxime (CTX) 30µg, Aztreonam (AT) 30 µg, Nitrofurantoin (NIT) 300µg, Erythromycin (E) 15g, Gentamycin (GN) 10µg, Tetracycline (TE) 30µg, Imipenem (IMP)10µg , Azithromycin (AZM) 15µg, Piperacillin (PI) 100µg, and Trimethoprim-Sulphamethoxazole (SXT) 1.25/23.75. A clear zone of more than 1 mm around a spot for probiotic spp. was considered as an indicator of antimicrobial effect [20]. All tests were replicated three times under the same identical experimental conditions.

2.3. Statistical analysis

The data obtained from three replicates were analyzed. Data (mean ± SD) were subjected to balanced ANOVA using Minitab v.16 and least significant differences (LSD) post cost testing. In all cases, the level of statistical significance was of $P < 0.01$ and $P < 0.001$ differences.

3. Results

On evaluation of the UTIs swabs, there were only 31 (73.8%) cases that showed positive bacterial culture with statistical differences (P<0.001) to negative bacterial culture 11 (26.2). Twenty three (74.2%) swabs were showed mixed growth colony with highly statistical differences (P<0.001) than single bacterial growth, eight (25.8 %), as outlined in Table 1.

Table 1. Distribution of percentage UTIs bacterial swabs.

	Negative Positive	Positive culture	
		Mixed culture	Single culture
Culture	11 (26.2%) 31 (73.8%)*	23 (74.2%)	8 (25.8 %)
Total	42	31	

***** Significant differences (P<0.001).

In addition, twenty four (77.42%) isolates were recorded. Therefore the frequencies of G-ve bacterial isolates were more than 24 (77.42%) and 7 (12.58%), respectively. Current results were highly significant (P<0.001), as outlined in Table 2. The results showed the recurrence of G-ve bacterial isolates of UT samples. *E coli* had a prevalent bacterial isolates 9(29.03%) with highly statistical differences (p<0.001), followed by *P mirabilis* 6 (19.36%), *K pneumonia* 5 (16.13%), and *P aeruginosa* 4 (12.90%). Forever, G+ve bacterial isolates demonstrated lower recurrence than G-ve bacterial isolates, with highly significant (p<0.001), where *S aureus* 5 (16.13%), and S epidermidis 2 (06.45%), as summarized in Table 2.

Table 2. Distribution of thirty one UT bacterial isolates.

Isolates	Number and percentage (%)	G-ve. bacteria	G+ve. bacteria
E coli	9 (29.03%)	9	0
P mirabilis	6 (19.36%)	6	0
S aureus	5 (16.13%)	0	5
K neumonia	5 (16.13%)	5	0
P eruginosa	4 (12.90%)	4	0
Sepidermidis	2 (06.45%)	0	2
Total	* 31 (100%)	24 (77.42%)	7 (12.58%)

***** Significant differences (P<0.001)

Furthermore, the current study demonstrated that the majority of infection with female 19 (61.3%) compare with male 12 (38.7%), as clarified in Table 3.

Table 3. Distribution of thirty one UT bacterial isolates on the sex of the patient.

Isolates	Female	Male
E coli	8 (88.9%)	1 (11.1%)
P mirabilis	3 (50%)	3 (50%)
S aureus	3 (60%)	2 (40%)
K pneumonia	2 (30%)	3 (70%)
P aeruginosa	3 (75%)	1 (25%)
S epidermidis	0 (0%)	2 (100%)
Total	*19 (61.3%)	12 (38.7%)

***** Significant differences (P<0.001).

Although, the infection was more with 0-9 and 20-29 years (6), 30-39 and 40-49 years (5), 10-19, 50-59, and 60-69 years (3), respectively, as outlined in Table 4.

On the other hand, the results with susceptibility antibiotic test for different bacterial isolates to lactobacilli spp., and antibacterial agents are showed in Table 5. *S aureus*, *S epidermidis*, and *P aeruginosa* were seen to have a high ability to resist all antibacterial agents with different percentages, and there were high significantly significant (P<0.0001), respectively. Moreover, the majority of pathogenic bacteria demonstrated high resistant, such as AMP AZM, CTX, PI, SXT, AT, and E, which were highly

Table 4. Distribution of thirty one UT bacterial isolates on the age categories (year).

*Ages (Y) / Isolates	0-9	10-19	20-29	30-39	40-49	50-59	60-69	Total
E coli	2	1	1	3	0	2	0	9
P mirabilis	0	2	1	0	1	1	1	6
S aureus	2	0	1	0	2	0	0	5)
K pneumonia	1	0	2	1	1	0	0	5
P aeruginosa	1	0	1	0	1	0	1	4
S epidermidis	0	0	0	1	0	0	1	2
**Total (%)	6	3	6	5	5	3	3	31

*(Y): Years, ** Significant differences (P<0. 01).

Table 5. Zones of inhibition (mm; mean ± SD) for UT isolates with probiotic *Lactobacillus* spp. broth culture bacteria (BCB).

Pathogens / Treatments	*E coli*	*P mirabilis*	*S aureus*	*K pneumonia*	*P aeruginosa*	*S epidermidis*
L fermentum	S (100)**	S (100)	S (100)	S (100)	S (100)	S (100)
L bulgaricus	S (100)	S (100)	S (100)	S (100)	S (100)	S (100)
TE	R (100)*	S (66.7)	R (60)	R (60)	R (75)	R (100)
AMP	R (100)	R (83.3)	R (100)	R (100)	R (100)	R (100)
AZM	R (88.9)	R (100)	R (100)	R (100)	R (83.3)	R (100)
IMP	S (77.8)	R (66.7)	R (80)	R (80)	R (80)	R (50)
GEN	S (88.9)	R (83.3)	R (80)	R (75)	R (75)	R (75)
AK	S (55.5)	R (75)	R (80)	R (60)	R (75)	R (100)
CTX	R (66.7)	R (83.3)	R (100)	R (80)	R (80)	R (100)
AT	R (88.9)	R (50)	R (100)	R (80)	R (83.3)	R (66.7)
SXT	R (77.8)	R (96.3)	R (100)	R (80)	R (96.3)	R (50)
PI	R (64.6)	R (96.3)	R (100)	R (100)	R (83)	R (100)
NIT	R (64.6)	S (66.7)	R (80)	S (60)	R (75)	R (50)
E	R (55.5)	S (66.7)	R (60)	R (80)	R (50)	R (100)

* Significant differences (P<0.01), **Significant differences (P<0.001).

significant (P<0.001), respectively. At same time, all pathogenic bacteria were highly sensitive (100%) to lactobacilli spp. and there were high significant differences (P<0.0001), as summarized in Table 5.
Generally, the results concluded that lactobacilli spp. showed a high activity to inhibit different of bacterial pathogenic growth *in vitro* of all pathogenic G-ve and G+ve bacteria, which cause UTIs. This indicates the therapeutic efficacy of probiotic lactobacilli spp.

4. Discussion

A main condition for lactic acid bacteria (LAB) with probiotic bacteria effectiveness is the productive capability of inhibitory substances that antagonize pathogenic isolates [21]. The current study is an important for a number of reasons. Unfortunately antibiotic therapy used to prevent recurrence of UTIs, there were no side effects and patients reported improved wellbeing and relief from their monthly cyclical recurrences of UTIs. All of UT isolates appeared sensitive to lactobacilli spp. compared with antibiotics activity, as outlined in Table 5. Probiotic *Lactobacillus* spp. indicated significantly greater (P < 0.001) inhibition zones against with all pathogen isolates. These results may be elucidated by the antimicrobial activity of various lactobacilli spp. through production of several antibacterial substances e.g. organic acids (LA, AA), bacteriocins, CO_2, H_2O_2 or others [22]. Pathogenic isolates were found to be sensitive to most *Lactobacillus* spp. This may be due to presence of greater levels of inhibitory substances in the bacterial culture broth, such as those associated with whole bacteria and antimicrobial substances. Cadieux *et al*. [18] demonstrated that lactobacilli spp. possess a high ability to inhibit pathogen growth and proliferation through competition with other pathogenic microorganisms for nutritional requirements. The results of the present study are compatible with other studies e.g. a study conducted by Tejero-Sarinena *et al*. [23], used an agar spot method to show that most of the selected isolates of probiotic lactobacilli, *Lactococcus*, *Bifidobacterium*, *Bacillus* and *Streptococcus* genera were able to produce active substances against causative agents, such as *S. aureus*. On the other hand, there are several lactobacilli spp., for example *L crispatus* and *L jensenii* demonstrated the ability to inhibit Gram positive cocci, such as *S aureus* growth and block sites adherence of this bacteria to HeLa cells *in vitro* [24]. Furthermore, the results of the current study demonstrate that the most infections in female (61.3%) compare with men (38.7%), as outlined in Table 3. These results were in agreement with the finding of Leigh [25], who observed that Incidence of UTIs are higher in women than men (40%- 50%) of whom will suffer at least one clinical episode during their lifetime. Furthermore, the current result also denstrated that UTIs increase with young compare with other ages (Table 4). These result compatible with the finding Raz [26], who reported that UTIs are the most common bacterial infection in young and women. Based on susceptibility tests, the current study compared lactobacilli spp. to numerous antibiotics that could be used locally or systemically in UTIs cases. Moreover, Table 5 demonstrated the susceptibility of bacterial isolates which have multidrug resistance to many antibiotics used with different percentages. All isolates appeared highly sensitive to *Lactobacillus* spp. with high significantly differences (P<0.001). These results interpret the antibacterial activity of lactobacilli through interaction with Toll-like receptor 2 (TLR-2), which recognizes bacterial lipoteichoic acid, lipoproteins, and other variance medical methods [15] production of several antibacterial substances e.g. H_2O_2, LA, AA, bacteriocins and others [17]. In addition, Cadieux *et al*. [18], and Hossain *et al*. [16] mentioned that lactobacilli have a high capability to inhibit pathogenic bacteria growth and multiplication via competition with other pathogenic microorganisms for nutritional requirements.

In the current study, Gram negative bacteria 24 (77.42%) were predominant compared with Gram positive; 7 (22.58%).

It is believed that these results are due to an opportunistic nature, and the ability to produce pus containing toxins (virulence factor). These toxins cause septicemia and interaction with immunity of patient, which leads to immunosuppressive cases. The high percentage 31 (73.8%) from positive bacterial culture of UTIs swab samples might be attributed to the fact that UTIs appear in high incidence as compared to other forms of infections. These symptoms facilitate invasion of UTIs pathogens. The reasons for the high incidence of *E coli* isolates might be due to factors associated with nosocomial causative agents with recurrent long term hospitalization that complicates illnesses, prior or random administration of antibacterial agents of UTIs [27]. Other pathogenic species of UTIs were segregated with low frequencies and various percentages because of their acquisition of nosocomial infection features and migration of these pathogens from GIT, wounds and respiratory tract to UT and immunosuppressant activities. Many studies have demonstrated that lactobacilli spp. play an important role in the protection of the UT ecosystem from opportunistic colonization by toxigenic causative agents and this could be attributed to two main mechanisms. These mechanisms involve the production of antimicrobial substances that cause opportunistic infective agents growth inhibition and the occlusion of pathogenic attachment to the UT epithelial cell walls. Although some lactobacilli serotypes demonstrate these urogenital mucosal protective features, significant differences among species and strains from a single species, are not uncommon [28]. In general, the agar spot method was a very effective method compared to other antimicrobial activity method.

5. Conclusion

The current study elucidates the antibacterial efficacy and immunological properties of probiotic lactobacilli spp. through investigation of UT pathogenic agents with susceptibility testing. We recommend the medical importance of *Lactobacillus* spp. in clinical applications for UTIs. In general, lactobacilli spp. can be applied as successful solutions to bacterial antibiotic resistance.

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