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Effect of *Lactobacillus acidophilus* on *Escherichia coli* causing Urinary tract infections in Vitro and in Vivo

Harith J. Fahad * **Alaa Muyassar Ahmed****

*University of Baghdad, College of Science,
**Technical Institute-Dohuk

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ABSTRACT

One hundred and sixty five mid stream urine specimens were collected from outpatients presented with urinary tract infections (UTI). The results showed the dominance of Escherichia coli over other causative agents. Antibiotic sensitivity test was carried out to E. coli isolates. Thence, the isolate that developed the highest multidrug resistance was chosen for further studies. Moreover, five Lactobacillus isolates comprising L. acidophilus L1 and L. acidophilus L2 were isolated from yogurt and vinegar, respectively, L. plantarum L3 and L. plantarum L5 from saliva and raw milk, respectively, while L. fermentum L4 was isolated from vagina. Cup assay method was employed to investigate the inhibitory (antagonistic) activity of lactobacilli isolates against E. coli A99 on MRS agar. Results showed that L. acidophilus L1 developed the highest activity. The cell free supernatant of lactobacilli developed the same activity. L. acidophilus L1 supernatant showed the highest inhibition activity. The present study also revealed this activity in vivo by injecting a group of mice with L. acidophilus L1 suspension or its infiltrate 30 min after injecting the E. coli A99 intraurethrally and the histopathological sections revealed the disappearance of inflammation signs caused by E. coli A99 when it was injected alone.

Introduction

Urinary tract infections are one of world- wide infections inflecting all age groups but more common in females. Escherichia coli is responsible for 80-90% of UTI cases (1).

Antibiotics participated in reduced UTI incidence (2). However, overuse of these antibiotics resulted in emergence of new strains have various strategies in multidrug resistance, and spread of super infections caused by these resistant microorganisms. This new emergence necessitates a need for novel and effective therapeutic strategies (3).

Probiotics are one of these successful strategies especially Lactobacillus metabolic by products which enhance the normal flora colonization eventually provide a protection against invading microorganisms (4, 5). Previously, we demonstrated the supernatant of Lactobacillus spp. lead to prevent the attachment of E. coli to uroepithelial cells in vitro (6) Therefore, this work aimed to cure UTI caused by E. coli using supernatant of Lactobacillus spp. in a murine model.

* Corresponding author at: University of Baghdad, College of Science.E-mail address:

Materials and Methods

In order to isolate E. coli, 165 mid stream urine specimens were collected from patients presented with UTI. Samples were transported to the laboratory and cultured on MacConkey agar plates at 37° C for 24 hrs. Identification was done according to the conventional methods (7,8).

To isolate lactobacilli, five different samples were collected from raw milk, vinegar, yoghurt, saliva and vagina, and streaked onto De Mann-Rogosa-Sharpe agar (MRSA) (pH 5.5)(Himedia, India) plates and incubated at 37 °C for 48 h under anaerobic conditions. The lactobacilli were initially identified by their ability to grow on the selective MRSA, gram-positive staining, rod shape, and catalase-negative phenotype. Biochemical analyses, including sugar fermentation profile and gas production in MRS broth (pH5.5) (Himedia, India), were conducted as described in Bergey's manual (9). Lactobacilli isolated from raw milk, vinegar, yoghurt, saliva and vagina were designated L1, L2, L3, L4 and L5, respectively.

Antibiotic susceptibility test

All E. coli isolates were tested for their

susceptibility toward ampicillin, cephotaxime, cephalexin, ciprofloxacin, gentamicin, nitrofurantoin, tetracycline and co-trimoxazole following the procedure of Bauer et al. (10). For quality control E. coli ATCC 25922 standard strain was tested as well.

Inhibitory effect of L. acidophilus supernatant on E. coli

in vitro study

Cup agar assay

Lactobacilli were cultured anaerobically on MRS agar for 48 hrs. at 37°C. Thereafter, 5 mm agar discs (triplicates) were cut out by a sterile Pasteur pipette and placed on a Muller Hinton agar (Himedia, India) plates seeded with E. coli A99. Then the plates were cultivated for 24 h. at 37° C and the inhibition zones were measured (11).

Overnight Lactobacillus spp. cultures contained 1.5 × 108 colony-forming at 37°C for 24 hr. were centrifuged at 6000 rpm/min for 10 min at 4°C. The resulting supernatants were filtered through a 0.2-µm membrane filter to remove the remaining bacteria and debris. All supernatants were cultured on MRSA in order to confirm the absence of lactobacilli cells (12,13). Thereafter, double fold serial dilutions were made from these supernatants and stored at 4 °C for assay.

Well diffusion method described by Ikeagwu et al. (14) was followed to detect Lactobacillus supernatant inhibition activity by spreading the highly resistant isolate; E. coli A 99 suspension (1.5 × 108 cfu/ml) over Mueller Hinton agar (HiMedia, India, pH7.2) plates using sterile cotton swab. Then 5 mm in diameter wells were cut out from the surface of previously cultured Mueller Hinton agar plate. Wells, in triplicates, were filled with 50 µl of Lactobacillus supernatant and MRS broth only. After an aerobic incubation at 37ºC for 24 hr., the diameter of inhibition zones caused by Lactobacillus supernatants, were measured.

The MIC values were defined as the lowest concentration inhibiting completely the bacterial growth (8).

in vivo study

Animals

Thirty five white female mice weighing 22-25 g were distributed into seven groups, five animals per group as following:

Groups A, B and C were injected intrurethrally by sterilized normal saline, sterilized MRS broth and L. acidophilus L1 supernatant which were considered as control groups. Groups D and E were injected with 1×108 cfu/ml of L. acidophilus L1and E. coli A99 cell suspensions, respectively. Group F was injected with 1×108 cfu/ml of E. coli A99 cell suspensions then after 30 min it was injected with 1×108 cfu/ml of L. acidophilus L1. Group G was injected with 1×108 cfu/ml of E. coli A99 cell suspensions then after 30 min it was injected with supernatant of L. acidophilus L1.

Injection protocol

First of all the bladder was emptied from urine by pressing on abdominal area. Urethra and surrounding area were sterilized with 75 % ethanol then a polyethylene tube (0.6 mm in diameter) was introduced to urinary bladder via urethra; the inoculums (20 µl) was injected by aid of this catheter. Thereafter, the catheter was withdrawn immediately, animals were returned to their cages with their lower end directed upward to avoid effusion of the inoculum outside (15).

All animals were kept in their cages without water for 24hrs. After 4 days of injection they were sacrificed and the left kidneys and bladders were aseptically removed, for histopathological study (16).

Results and Discussion

One hundred and fifty specimens were positive for bacterial culture. E. coli formed 51.3% of total positive specimens. Among those, 66% were female and 34% were male patients.

Results present in figure 1 demonstrate that all E. coli isolates were resistant to cephalexin; while nitrofurantoin recorded the lowest resistance percentage.

The E. coli A99 isolate developed the highest multidrug resistance; hence it was chosen for further experiments.

Results of lactobacilli identification showed that the five isolates belong to three species; L1 and L2 were L. acidophilus, L3 and L5 were L. plantarum; whereas, L4 was identified as L. fermentum.

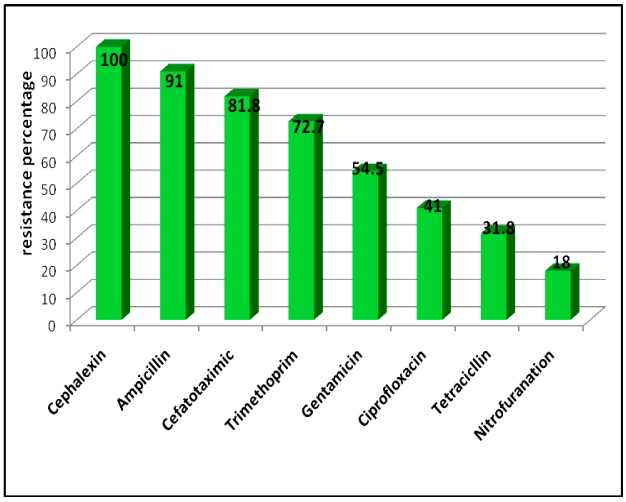

Figure 1: Antibiotic resistance of *E. coli* isolated from Iraqi UTI patients

Inhibitory effect of lactobacilli against E. coli A99

Table 1 illustrates a remarkable inhibitory effect of lactobacilli supernatants against test isolate; E. coli A99. There were no significant differences (P>0.05) among inhibitory effects neither among cells co-culture nor among supernatants under speculation. Nevertheless, highest inhibition diameter was achieved by L. acidophilus L1 which was isolated from yogurt; consequently, it was used in in vivo experiments.

Table 1: Inhibitory effect of lactobacilli supernatant on *E. coli* A99.

***Lactobacillus* species**	**code**	**Source of isolation**	**Inhibition zone diameter ± SD***	
			Agar cup	**Supernatant**
L. acidophilus	**L_1**	**Yogurt**	**20.6 ± 1.2**	**26.3 ± 1.5**
L. acidophilus	**L_2**	**Vinegar**	**19 ± 1.0**	**22.6 ± 1.2**
L. plantarum	**L_3**	**Saliva**	**18.3 ± 0.6**	**20.6 ± 1.5**
L. fermentum	**L_4**	**Vagina**	**15.3 ± 1.5**	**15.3 ± 1.5**
L. plantarum	**L_5**	**Raw milk**	**17.6 ± 0.6**	**20.6 ± 1.2**
***P* value**			**0.841412**	**0.575916**

***SD= standard deviation.**

in vivo study

Figure 2 shows the normal structure of kidney and urinary bladder of mice injected intraurethrally with normal saline or sterile MRS broth.

While injecting the mice with 1×108 cfu/ml of E. coli A99 caused several histopathological changes in urinary bladder involved infiltration of inflammatory cells and increase in caliber and number of blood vessels; whereas kindney showed infiltration of inflammatory cells in addition to shrinkage of glomeruli and increase in interstitial space as it shown in figure 3.

Virulence factors of E. coli, can induce many of the host defenses required for bacterial killing causing increase in cytokines, influx of neutrophils and induction of iNOS (17).

Concerning the in vitro success in inhibiting E. coli growth, the inflammatory signs disappeared after treatment with either L. acidophilus cells or supernatant (figure 4).

Such curing action of lactobacilli could be attributed to their antimicrobial products such as bacteriocins, hydrogen peroxide, organic acids and many other materials (3). Also it could be assigned to their ability to inhibit the attachment of pathogens to uroepithelial cells (6).

It was concluded that the present work signifies the probiotic role of either cell suspension or cell free supernatant of L. acidophilus in elimination the UTI infection caused by E. coli.

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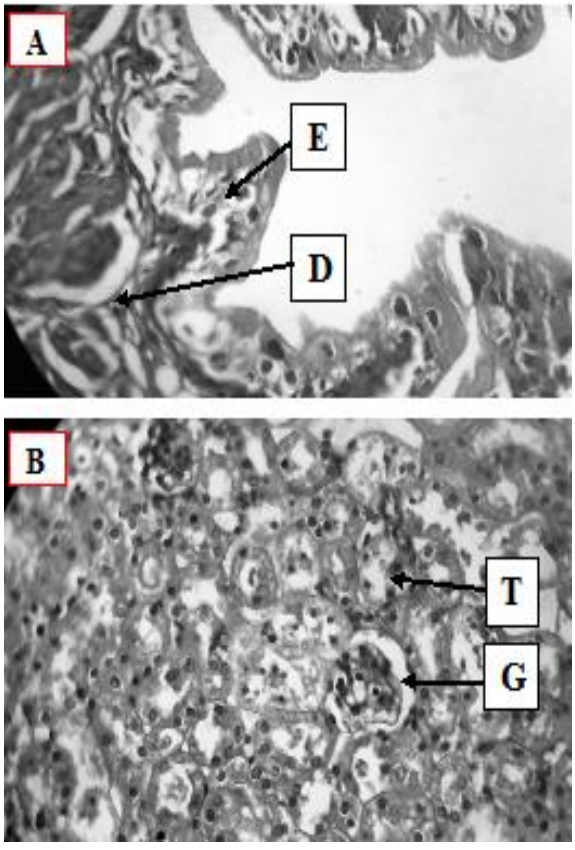

Figure 2: Cross section in normal urinary bladder (A) and kidney (B) of mice injected with normal saline. G= glomeruli, T= tubules, epithelial layer (E), dermis layer (D). X400. H&E.

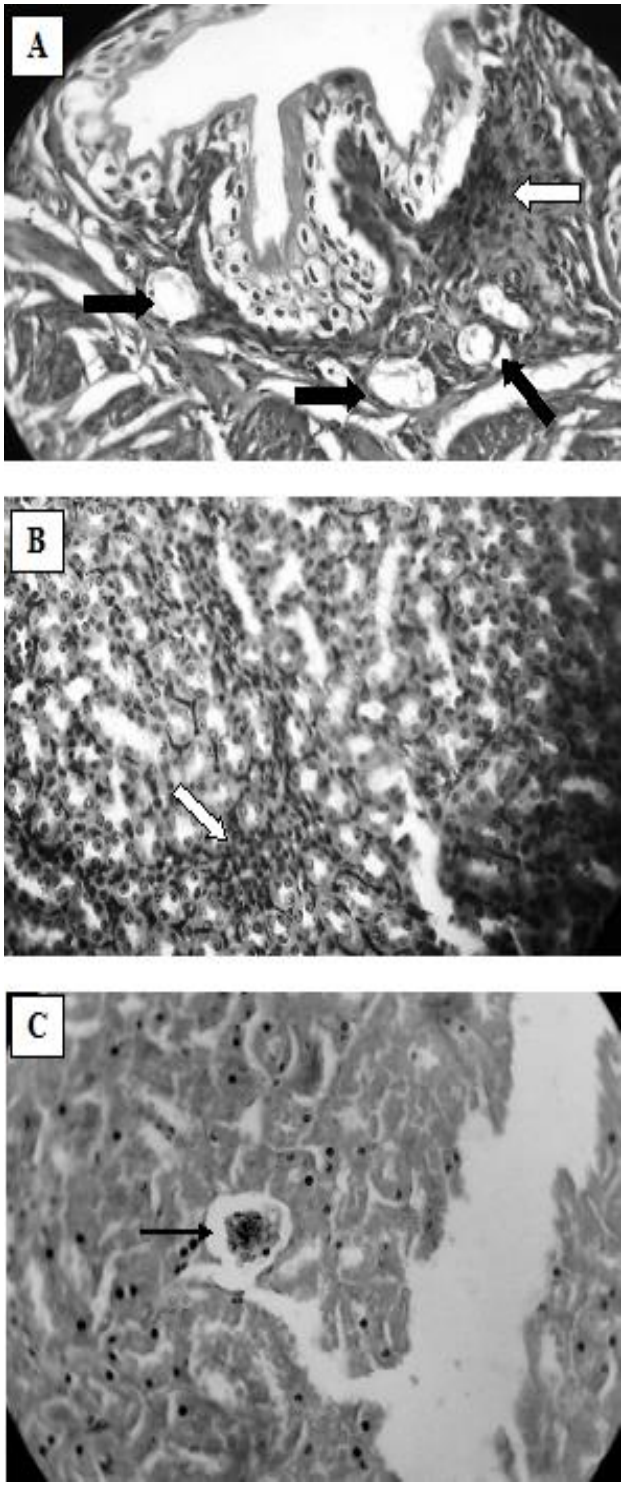

Figure 3: Cross section in normal urinary bladder (A) and kidney (B,C) of mice injected with 1×10^{8} cfu/ml of *E. coli* A99 shows infiltration of inflammatory cells (white arrow) and increase in caliber and number of blood vessels (black arrows) shrinkage of glomerulus (small arrow). X400. H&E.

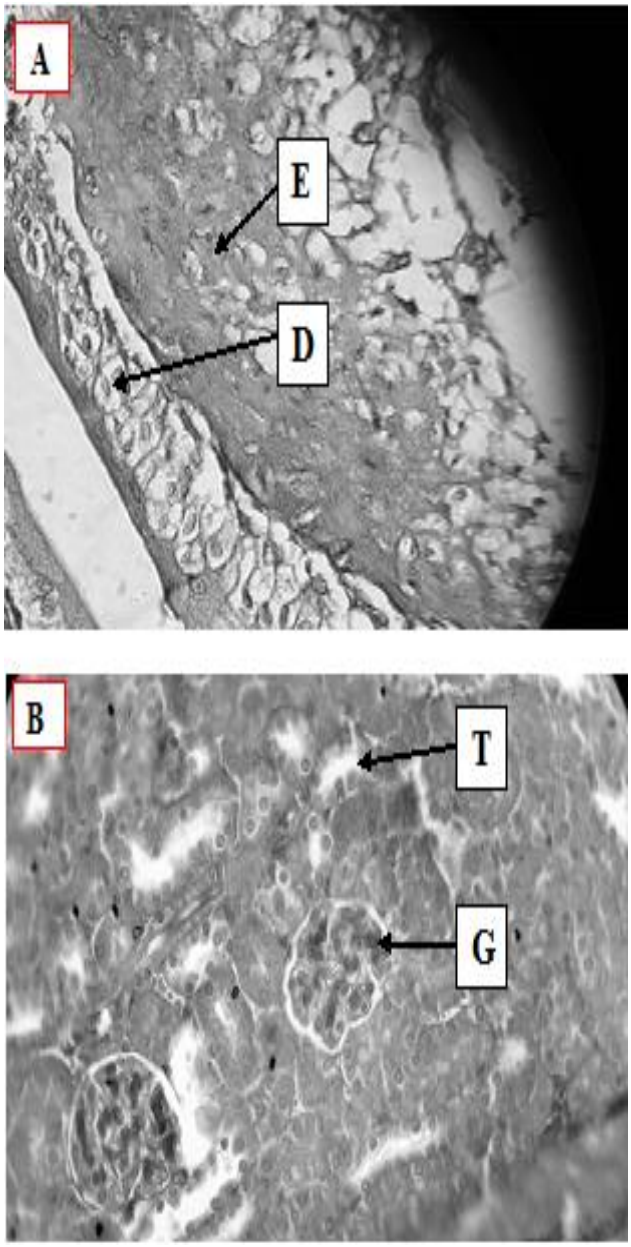

Figure 4: Cross section in urinary bladder (A) and kidney (B) of mice injected with 1×108 cfu/ml of E. coli A99 then after 30 min it was injected with 1×108 cfu/ml of L. acidophilus L1. G= glomeruli, T= tubules, epithelial layer (E), dermis layer (D). X400. H&E.

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تأثير جرثومة الحليب الحامضية *Lactobacillus acidophilus* في الايشريشية القولونية *E. coli* المسببة لخمج المجاري البولية داخل و خارج الجسم الحي

حارث جبار فهد **الاء ميسر احمد**

الخلاصة

جمعت 165 عينة ادرار وسط المجرى من مرضى مصابين بخمج المجاري البولية. اذ اظهرت نتائج الدراسة الحالية سيادة Escherichia coli على بقية المسببات المرضية. درست الحساسية للمضادات الحياتية لهذه الجراثيم و انتخبت العزلة الاكثر مقاومة لدراسات لاحقة. كما عزل خمس عزلات من جرثومة Lactobacillus مثلت L. acidophilus L1 و L. acidophilus L2 المعزولة من اللبن و الخل على التوالي و L. plantarum L5 و L. plantarum L4 المعزولة من اللعاب و الحليب الخام على التوالي في حين عزلت L. fermentum L4 من المهبل. استعملت طريقة كأس الاغار للتحري عن الفعالية التثبيطية لعزلات Lactobacillus ضد العزلة E. coli A99 على الوسط الزرعي MRS اذ بينت النتائج ان L. acidophilus L1 اظهرت اعلى فعالية. علاوة على ذلك اظهر طافي خلايا Lactobacillus فعالية تثبيطية مشابهه في حين اظهر طافي L. acidophilus L1 الفعالية الاكبر. كما بحثت الدراسة الحالية هذه الفعالية داخل الجسم الحي عن طريق حقن مجموعة من الفئران بعالق خلايا L. acidophilus L1 او طافي تلك الخلايا بعد 30 دقيقة من حقن E. coli A99 عن طريق الاحليل واخذت الكلى و المثانة للدراسة النسيجية التي بينت اختفاء علامات التهاب التي تسببت بها E. coli A99 عندما حقنت لوحدها.