Probiotics: Lactic Acid Bacteria have Antibacterial Activity and Downregulate Biofilm Genes of Uropathogenic E. coli

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Abstract

Urinary tract infections (UTIs) are regarded as one of the most serious infections worldwide. Uropathogenic E. coli (UPEC) accounts for nearly 80% of UTI infections in females. This study investigated the antibacterial and antibiofilm effects of Lactobacillus acidophilus (L. acidophilus) and Lactobacillus plantarum (Lb. plantarum) on multidrug-resistant E. coli obtained from urine samples. Complete bacteriological identification was conducted on 45 E. coli isolated from 80 urine samples of females with UTIs. Antibiotic susceptibility test was performed on all isolates by nine antibiotics. Ten out of the 45 isolates exhibited multidrug resistance (MDR). L. acidophilus and Lb. plantarum showed marked inhibition of MDR E. coli isolates on agar by a diffusion method (16 ± 0.04: 23 ± 0.05 mm). Moreover, L. acidophilus and Lb. plantarum strains inhibited the ability of UPEC to form a biofilm by 56.3% and 39.63%, respectively. The expression of biofilm genes of E. coli are as follows: csgA, crl, csgD showed remarkable downregulation after treatment with probiotics suspension: 0.00364: 0.19078 fold, 0.0005: 0.1894 fold, and 0.0490: 0.0883 fold for L. acidophilus, respectively. On the other hand, downregulation of biofilm gene expression for csgA, crl, csgD after treatment with Lb. plantarum suspension were expressed by fold changes as follows: 0.0769: 0.3535 fold, 0.05440: 0.12940 fold, and 0.06745: 0.4146, respectively. These findings show that L. acidophilus and Lb. plantarum exhibit potent antibacterial and antibiofilm action against MDR UPEC at both genotypic and phenotypic levels, and appear to be a promising solution in therapeutic applications for recurrent and persistent UTIs.

Keywords: Urinary Tract Infections (UTIs), Uropathogenic E. Coli, Antibacterial, Antibiofilm, Lactobacillus acidophilus, Lactobacillus Plantarum

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INTRODUCTION

Enterobacteriaceae are a common intestinal flora of the digestive system in humans, but this type of bacteria can cause extraintestinal urinary tract infections (UTIs). The misuse of antibiotics has led to the increased occurrence of resistant isolates worldwide. Nowadays, the emergence of MDR E. coli has contributed to a significant problem, as there are limited therapeutic options for these pathogens, therefore leading to increased levels of both morbidity and mortality. There is an imperative need for new alternative therapeutics to treat the emergence of MDR E. coli.

UPEC biofilm is responsible for persistent infection and resistance to antibiotics. Biofilms are groups of microorganisms that may be mono-or multispecies, embedded in a matrix of extracellular polymeric substances (EPS). This matrix is composed of exopolysaccharides, proteins, DNA, RNA, lipids, and signaling molecules (e.g., autoinducers), which enable communication to occur between bacterial cells through a phenomenon known as quorum-sensing (QS). The biofilm matrix acts as a block for the entry of chemical agents, immune molecules, and pH changes in the surrounding environment. In addition, the matrix promotes antibiotic resistance and facilitates the spread of resistant genes. Many genes encode for the process of E. coli biofilm formation.

Curli, a kind of amyloid fimbriae, aids the adherence to the urinary bladder and biofilm development. Curli fimbriae assist in cell adhesion, biofilm development, and aggregation in E. coli to a surface. The main curli subunit csgA, which is important for adhesion to host components, is encoded by the csgBAC operon. The csgA gene was demonstrated to cause extremely drug-resistant UPEC in clinical isolates. Curli acts as a glue to adhere the bacterial cells to numerous serum proteins and the extracellular matrix.

Several non-antibiotic approaches have recently emerged, such as the use of immunomodulators, herbal extracts, hormonal, and biological therapeutics like probiotics. Probiotics offer many advantages over other therapeutics, as they are generally recognized as safe (GRAS). Lactobacilli are important members of the probiotic family. Lactobacilli bacteria demonstrate many antimicrobial mechanisms, including competition with pathogenic bacteria for their binding sites and nutrition, stimulation of the protective immune response, secretion of inhibitory molecules such as hydrogen peroxide, fatty acids, bacteriocins, and ethanol. In addition, Lactobacilli can produce many types of acids that reduce intestinal pH, for instance, acetic acid, lactic acid, and formic acid. Lactobacilli have demonstrated their capability to act against Pseudomonas aeruginosa, Shigella spp., E. coli, Clostridium difficile, Staphylococcus aureus, and Streptococcus mutants. However, few studies have studied the lactobacilli activity towards MDR Uropathogenic E. coli. Thus, we conducted the present study to discover the different antibacterial and antibiofilm abilities of lactobacilli against UPEC isolates at both phenotypic and genotypic levels.

MATERIALS AND METHODS

Microbiological Examination and E. coli Isolation

This study was carried out on 80 female patients exhibiting UTIs, aged 23 to 58 years, recruited from the inpatient department and outpatient clinic of the internal medicine department, Faculty of Medicine for Girls, Al-Zahraa Hospital—Al Azhar University, Cairo, Egypt, during the period of November 2020 to January 2021.

80 urine samples were aseptically obtained and immediately relocated to the microbiology laboratory. MacConkey agar (Oxoid, USA) was used to culture the samples, which were then incubated aerobically for 24 hours at 37°C. 45 E. coli isolates were obtained. The isolated colonies were then fully identified by complete biochemical identification. All E. coli strains were kept at −20°C in a growth medium containing glycerol. Probiotics, L. Acidophilus and Lb. plantarum reference strains (ATCC 4356, ATCC 14917) were obtained from the Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Egypt. Both lactobacilli strains were cultured on Man-Rogosa- Sharpe (MRS) (HiMedia, India) agar and broth medium under anaerobic conditions at 37°C for 48 h with 5% CO₂.
Antibiotic Sensitivity Test for Isolated E. coli

Susceptibility testing for all E. coli isolates was carried out according to the disk diffusion (modified Kirby Bauer) assay on Mueller–Hinton agar (Merk, Germany) in accordance with the Clinical and Laboratory Standard Institute (CLSI) guidelines in the case of the following antibiotics (Oxoid, UK): Amoxicillin + Clavulanic Acid (AMC 20 + 10 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CRO, 30 µg), Ciprofloxacin (CIP 5 µg), Amikacin (AK 30 µg), Cefotaxime (CTX 30 µg), Ampicillin (AMP 10 µg), Gentamycin (CN 30 µg), and Nitrofurantoin (F). The results were inferred utilizing CLSI guidelines (2019).

Agar Well Diffusion Method for MDR Isolates

All MDR isolates were further subjected to the agar diffusion method to assess the antibacterial actions of *L. acidophilus* and *Lb. plantarum*. Briefly, the suspension of *E. coli* bacteria was adjusted to half McFarland and cultured on nutrient agar plates. 100 µL of each probiotic (0.5 McFarland turbidity) was spilled into 6 mm wells, which were then cut with sterile tips in the plates. After incubation for 24h at 37°C, the size of inhibitory zones diameters was determined in millimeters using a ruler.

Antibiofilm Assay

Probiotic isolates were further tested for their antibiofilm activity. A single colony of each *E. coli* isolate was added to 5 mL of nutritional broth (Oxoid, UK) and cultured for 20 hours at 37°C. The antibiofilm formation activity was tested as described by Jadhav et al. The two tested probiotics, alongside their control (broth medium), were put in a 96-well plate (Sigma Aldrich, USA).

10% (v/v) of all probiotics were used as recommended by Medellin-Pena et al. 40 µL of the tested probiotics were added to the wells in triplicate, except for the negative controls (40µL of broth medium). For each group, 160 µL of *E. coli* broth cultures were added (broth medium was added by the same volume to control wells instead), reaching an ultimate volume of 200 µL per well. Then, the microtiter plates were closed and incubated at 37°C for 24 h. After incubation, the culture medium was removed, and then all wells were rinsed three times with sterile distilled water to eliminate any attached cells. After allowing the microtiter plate to air dry, it was dyed with 150 µL of 0.1 percent crystal violet. To remove any unabsorbed stain, the stain was allowed at room temperature for 15 minutes before being washed twice with sterile distilled water. To solubilize the crystal violet, ethanol was applied to all wells. The test organisms’ mean optical density absorbance was determined at 595 nm, and the percentage of inhibition was computed using the formula (Eq.):

\[
\text{Percentage inhibition} = 100 - \left( \frac{\text{OD}_{595 \text{ nm test for positive control well}}}{\text{OD}_{595 \text{ nm test for negative control well}}} \right) \times 100
\]

Polymerase Chain Reaction (PCR) Amplification and DNA Extraction

DNA was collected from the three *E. coli* isolates most affected using the QIAamp DNA Mini kit (Germany, Qiagen, GmbH). The isolates were examined for the prominence of biofilm genes *crl*, *csgA*, and *csgD* as mentioned in Table 1.

Agarose Gel Electrophoresis of PCR Products

By electrophoresis on a 1% agarose gel (Applichem, Germany, GmbH) with 20 µL of PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>csgA</td>
<td>ACTCTGACTTGACTATATTACC AGATCGAGTCGTCAGCAAC</td>
<td>200 bp</td>
<td>19</td>
</tr>
<tr>
<td>crl</td>
<td>TTTGAGATTGCTTGCGGCTATG GCTTGCATTACGGGTCGTC</td>
<td>250 bp</td>
<td>19</td>
</tr>
<tr>
<td>csgD</td>
<td>TTATCCGCTGAGTATGCAGCAGGCAGGTCGTC</td>
<td>501 bp</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 1. Different primers and its sequences used for detecting biofilm genes
products in each well, the PCR products were split up. A gel documentation system was then used to visualize the gel (Alpha Innotech, Biometra).

**qRT-PCR (Quantitative, Reverse Transcriptase PCR) for Biofilm Genes**

The expression of biofilm genes was analyzed using qRT-PCR. The 16S rRNA housekeeping gene was used as an internal control to ensure that the expression levels of the samples were comparable. The total reaction volume of 25 μL contained 0.25 μL of RevertAid Reverse Transcriptase (200 U/μL) (Thermo Fisher), 0.5 μL of each primer of 20 pmol concentration, 12.5 μL of the 2x QuantTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 8.25 μL of water, and 3 μL of cDNA template. The reaction was carried out in a Step-one real-time PCR according to the settings listed in Table 2. Amplification curves, as well as Ct values, were evaluated. The variance of gene expression on the RNA of the various samples was assessed, and the Ct of each sample was compared to that of the positive control group using the "ΔΔCt" protocol investigated by Yuan et al.21

**RESULTS**

**Biochemical Identification of UPEC Isolates and Antibiotic Susceptibility Test**

The collected isolates produced rose pink colonies when grown on MacConkey agar due to lactose fermentation. All were confirmed as *E. coli* bacteria by the exhibited biochemical reactions, including methyl red and indole positive, urea and citrate negative, and Motility Indole Ornithine positive. From the 80 urine samples, 45 *E. coli* isolates were obtained. The characteristics of the female patients who took part in this study are summarized in Table 3. The disc diffusion method was used to test all isolates with nine antibiotics (CRO, AMP, CN, F, CIP, CXM, AK, AMC, CAZ). The antibiotic susceptibility pattern of all isolates is summarized in Table 4. Ten isolates showed multidrug-resistant UPEC to at least one antibiotic in three or more antimicrobial categories. The antibiotic susceptibility pattern of MDR isolates is summarized in Table 5.
Antibacterial Activity of *L. acidophilus* and *Lb. plantarum* against MDR Isolates

Eight MDR *E. coli* isolates gave sensitivity to *L. acidophilus*, and seven out of ten to *Lb. plantarum*. The inhibition zones range from (16±0.04–23±0.05 mm). The inhibition zones for the different isolates are captured in Table 6.

Antibiofilm Assay Results for the MDR *E. coli* Isolates

The effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* on *E. coli*’s preliminary adhesion to biofilm development was observed and reported in Table 7. Overall, the majority of the *E. coli* isolates tested demonstrated a sufficient ability to create biofilms. *L. acidophilus* induced the highest inhibition (56.30%) in case of *E. coli* 44, followed by 45.63% - *E. coli* 8 and 43.20% - *E. coli* 42. While *L. plantarum* caused 39.63% reduction of *E. coli* 8 biofilm. *L. plantarum* had a minor inhibitory effect on *E. coli* 42 and *E. coli* 8 isolates (inhibition percentages of 22.68 and 28.84, respectively). In contrast, *E. coli* 32 biofilm was negatively influenced by both probiotics (Table 7). Based on the previous results, *E. coli* 8, *E. coli* 42, *E. coli* 44 were used to detect the influence of tested probiotics on the genetic level.

Detection of Biofilm Genes Using Conventional PCR

Tested *E. coli* strains carried *crl*, *csgA*, and *csgD* genes and produced bands at 250, 200, and 501 bp, respectively, as shown in Figure 1.

Quantitative Assessment Effect of Probiotic Bacteria on Biofilm Genes of Tested *E. coli* Isolates Using qRT-PCR

Data in Figure 2 shows the expression

### Table 4. Antibiotic susceptibility results for all *E. coli* isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Conc. (µg/disc)</th>
<th>Resistant (R) No.* (%#)</th>
<th>Intermediate(I) No* (%#)</th>
<th>Susceptible (S) No* (%#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin (CN)</td>
<td>10</td>
<td>16 (35.5)</td>
<td>3 (6.5)</td>
<td>26 (58)</td>
</tr>
<tr>
<td>Ampicillin (AMP)</td>
<td>10</td>
<td>26 (57.7)</td>
<td>1(2.3)</td>
<td>18 (40)</td>
</tr>
<tr>
<td>Amikacin (AK)</td>
<td>30</td>
<td>7 (15.5)</td>
<td>13 (29)</td>
<td>25 (55.5)</td>
</tr>
<tr>
<td>Cefuroxime (CXM)</td>
<td>30</td>
<td>17 (37.8)</td>
<td>8(17.8)</td>
<td>20 (44.4)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>5</td>
<td>8 (17.8)</td>
<td>5(11.1)</td>
<td>32(71.1)</td>
</tr>
<tr>
<td>Nitrofurantoin (F)</td>
<td>300</td>
<td>11(24.4)</td>
<td>14(31.2)</td>
<td>20(44.4)</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>30</td>
<td>18(40)</td>
<td>14(31)</td>
<td>13(29)</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>30</td>
<td>13(29)</td>
<td>5(11)</td>
<td>27(60)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>20</td>
<td>33(73.3)</td>
<td>4(9)</td>
<td>8(17.7)</td>
</tr>
</tbody>
</table>

#expressed as percent regarding all *E.coli* isolates for each antibiotic tested.

*Designates number of *E.coli* isolates.

*Denotes: (R) Resistant, (I) Intermediate, (S) Susceptible.

### Table 5. The most resistant *E.coli* isolates to different antibiotics

<table>
<thead>
<tr>
<th>Code</th>
<th>CN</th>
<th>AMP</th>
<th>AK</th>
<th>CXM</th>
<th>CIP</th>
<th>F</th>
<th>CAZ</th>
<th>CRO</th>
<th>AMC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> 1</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>E. coli</em> 6</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>E. coli</em> 8</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>E. coli</em> 9</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>E. coli</em> 16</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>E. coli</em> 31</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>E. coli</em> 32</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>E. coli</em> 42</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> 34</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> 44</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>

S: sensitive, R: resistant, I: intermediate.
of the investigated biofilm gene products (cDNA) before and after treatment with probiotic solutions.

The fold changes in csgA, crl, csgD gene expression after treatment with L. acidophilus suspension were remarkably downregulated: 0.00364: 0.19078 fold, 0.0005: 0.1894 fold, and 0.0490: 0.0883, respectively. On the other hand, downregulation of biofilm gene expression for csgA, crl, csgD after treatment with L. plantarum suspension were expressed as fold changes: 0.0769: 0.3535 fold, 0.05440: 0.12940 fold, and 0.06745: 0.4146, respectively.

**DISCUSSION**

UPEC is a common cause of UTIs. In the present study, ten E. coli isolates were considered to be resistant to several classes of antibiotics. MDR pathogens are considered a serious threat to human health due to their severity and spreading capabilities. The rising antibiotic resistance of UPEC due to the misuse of antibiotics and the biofilm ability of E. coli entail a need for alternative treatment options. As previously published, E. coli resistance to many antibiotics like amoxicillin, clarithromycin, ampicillin, ceftriaxone, ceftazidime, and clavulanic acid) were reported by Abdelhamid et al. Also, high percentage of E. coli strains (60.6%) showed resistance to trimethoprim/sulfamethoxazole, ampicillin, tetracycline, and cefazolin. Therefore, the emergence of MDR and antibiotic resistance in recent times has led to the development of new alternatives to combat the MDR bacteria. Probiotics are a necessary emerging alternative that contains antimicrobials and antibiofilm properties.

Probiotics have an inhibitory ability against many pathogenic bacteria both in vitro and in vivo. In this study, the antimicrobial activity of two lactobacilli strains, L. acidophilus and Lb. plantarum, was examined for their potential to

![Figure 1](image1.png)

**Figure 1.** Agarose gel electrophoresis for crl, csgA, and csgD genes of three E. coli isolates giving bands at 250, 200, and 501 bp, respectively.

![Figure 2](image2.png)

**Figure 2.** Results of RT-PCR showing the expression of csgA, crl, and csgD in E. coli sp. before and after treatment with Lactobacillus acidophilus and Lactobacillus plantarum.
inhibit the growth of MDR E. coli by utilizing the agar well diffusion method. Eight out of ten MDR E. coli isolates showed sensitivity to L. acidophilus, and seven out of ten to Lb. plantarum. Dawwam et al.\textsuperscript{25} found that this inhibition potential due to the presence of different bioactive compounds having antimicrobial activities as 9-Octadecenoic acid, Oleic acid, 2,2- Dideutero octadecanal, 1-Hexadecanol, 2-methyl. These compounds were detected in both extracts of L. plantarum and L. Acidophilus using GC-Ms spectroscopy.

Similar results were obtained by Hashem and Abd El-Baky\textsuperscript{10} who found that all tested E. coli isolates showed high sensitivity to Lactobacilli supernatants. Moreover, in a study conducted by Karigoudar et al.,\textsuperscript{28} reported that 89.7% of UPEC isolates among the catheterized patients were biofilm-producing. According to previous studies, probiotics demonstrated their ability to reduce biofilm formation. In the study conducted by Hashem and Abd El-Baky,\textsuperscript{10} the researchers found that 16 out of 22 cell-free spent media (CFSM) of Lactobacillus isolates from healthy infants (aged 3–6 months), showed a 50% reduction in biofilm formation.

### Table 6. Antibacterial activity of L. acidophilus and L. Plantarum against MDR isolates

<table>
<thead>
<tr>
<th>E.coli Code</th>
<th>L.Plantarum M (SD)</th>
<th>L. Acidophilus M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 1</td>
<td>0±0.04</td>
<td>18±0.04</td>
</tr>
<tr>
<td>E. coli 6</td>
<td>20±0.08</td>
<td>18±0.13</td>
</tr>
<tr>
<td>E. coli 8</td>
<td>18±0.14</td>
<td>20±0.08</td>
</tr>
<tr>
<td>E. coli 9</td>
<td>19±0.06</td>
<td>19±0.05</td>
</tr>
<tr>
<td>E. coli 16</td>
<td>16±0.04</td>
<td>18±0.14</td>
</tr>
<tr>
<td>E. coli 31</td>
<td>0±0.12</td>
<td>0±0.0</td>
</tr>
<tr>
<td>E. coli 32</td>
<td>22±0.06</td>
<td>18±0.05</td>
</tr>
<tr>
<td>E. coli 42</td>
<td>0±0.0</td>
<td>0±0.0</td>
</tr>
<tr>
<td>E. coli 34</td>
<td>23±0.05</td>
<td>22±0.06</td>
</tr>
<tr>
<td>E. coli 44</td>
<td>18±0.06</td>
<td>16±0.09</td>
</tr>
</tbody>
</table>

Legend—M: mean expressed in mm; SD: standard deviation.

### Table 7. Antibiofilm activity of probiotics against MDR E. coli isolates

<table>
<thead>
<tr>
<th>E.coli code</th>
<th>OD595nm (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>E. coli 1</td>
<td>0.1224±0.08 (24.02)</td>
</tr>
<tr>
<td>E. coli 6</td>
<td>0.1322±0.06 (8.19)</td>
</tr>
<tr>
<td>E. coli 8</td>
<td>0.1296±0.008 (45.63)</td>
</tr>
<tr>
<td>E. coli 9</td>
<td>0.1541±0.07 (27.92)</td>
</tr>
<tr>
<td>E. coli 16</td>
<td>0.1986±0.05 (41.12)</td>
</tr>
<tr>
<td>E. coli 31</td>
<td>0.1655±0.06 (0) *</td>
</tr>
<tr>
<td>E. coli 32</td>
<td>0.3044±0.12 (0) *</td>
</tr>
<tr>
<td>E. coli 42</td>
<td>0.1426±0.09 (43.20)</td>
</tr>
<tr>
<td>E. coli 34</td>
<td>0.1684±0.08 (29.36)</td>
</tr>
<tr>
<td>E. coli 44</td>
<td>0.1421±0.05 (56.30)</td>
</tr>
</tbody>
</table>

* Means that when the biofilm biomass of treated E. coli is equal to or greater than the control, there is no inhibition.
consisting of the UPEC isolates. In addition, an 80% reduction was observed in four Lactobacillus isolates.

The study of Abdelhamid et al. showed that the biofilms formed by MDR E. coli isolates were reduced by L. helveticus and Lb. plantarum CFS (69.49% and 64.57%), respectively. Furthermore, Aboulwafa et al. proved the antibiofilm potential of some Lactobacillus strains against E. coli, S. aureus, and Pseudomonas aeruginosa.

According to our study, E. coli isolates harbored csgA, crl, csgD genes. In this regard, Luna-Pineda et al.; Ochoa et al. showed that UPEC clinical strain obtained from pediatric patients with UTIs was found to contain a high proportion (95%) of the csgA gene. In addition, Sharma et al.; Ikwap et al. mentioned that crl and csgA genes regulate the surface factors of curli fimbriae, which have an important role in mediating the production of exopolysaccharide (EPS) and adhesion of E. coli to biotic and abiotic surfaces.

Lactobacillus strains exhibit various mechanisms that result in a positive effect against pathogens. According to our data, the fold changes in csgA, crl, csgD gene expression after treatment with L. acidophilus suspension were remarkably downregulated than Lb. plantarum. Song et al. established that L. rhamnosus L. rhamnosus cells on microcapsules reduced the transcriptional activity of some virulence genes responsible for the regulation of E. coli Qs such as luxS, lsrK, and lsrR, and hence reduce the biofilm formation of E. coli.

Also, L. rhamnosus and L. salivarius significantly downregulated the gene expression of some Streptococcus mutans virulence genes as glucosyltransferases (gtfD, gtfB, and gtfC), which are responsible for glucan biosynthesis and biofilm formation. Moreover, Matsubara et al. and Rossoni et al. revealed that L. acidophilus, L. fermentum, L. paracasei, and L. rhamnosus downregulated the genes involved in biofilm development, and gluconeogenesis and glycolysis of C. albicans. Another study by Qian et al. discovered that the culture supernatant of Lactobacillus sp. reduced the biofilm formation of Gardnerella vaginalis, which is responsible for bacterial vaginosis. Lb. plantarum ZX27 supernatant decreased the expression of genes responsible for biofilm formation, virulence factors, adhesion, metabolism, and antimicrobial resistance. The potential role of probiotics in reducing microbial biofilm by enabling growth inhibition, bacteriocin production, co-aggregation, and adhesion are readily observed.

**CONCLUSION**

Our results support that L. acidophilus and Lb. plantarum have prominent probiotic activity, exhibiting both antibacterial and antibiofilm effects against MDR UPEC at both phenotypic and genotypic levels by downregulating the biofilm encoding genes. This reinforces their use as an alternative non-antibiotic therapy, especially against MDR isolates in recurrent UTIs. Further in vitro and in vivo studies on a larger scale analyzing other probiotic strains remain necessary.

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None.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHORS’ CONTRIBUTION**

GED, HFI and MHY designed and directed the project. GED, HFI and IIS performed the Experiments. GED, HFI analyzed the data. GED, HFI wrote the Initial draft. GED reviewed and edited the final manuscript. All authors read and approved the final manuscript for publication.

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**DATA AVAILABILITY**

All datasets generated or analyzed during this study are included in the manuscript.
ETHICS STATEMENT

Not applicable.

REFERENCES


