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Probiotic Inhibition of Uropathogenic *Escherichia coli* in Pediatric Urinary Tract Infections: Antibiofilm and Antimicrobial Effects of *Lactobacillus reuteri* and *Lactobacillus plantarum*

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Abstract. *Escherichia coli is the predominant pathogen in pediatric urinary tract infections (UTIs), yet increasing antimicrobial resistance, virulence gene carriage, and biofilm formation complicate therapeutic success. Probiotic-derived metabolites represent a potential complementary strategy to mitigate persistence of multidrug-resistant (MDR) uropathogens; however, data in pediatric UTIs remain scarce. To determine the antimicrobial resistance pattern, biofilm-forming ability, and virulence gene prevalence among E. coli isolates from pediatric UTIs, and to evaluate the antibacterial and antibiofilm activities of cell-free supernatants (CFS) of Lactobacillus plantarum and Lactobacillus reuteri.*

Methods. One hundred urine samples from children aged 1 month to 10 years were analyzed. Bacterial identification was performed using culture, biochemical profiling, and VITEK. Antibiotic susceptibility was tested using the Kirby–Bauer method. Biofilm formation was quantified via crystal violet assay, and the fimH and ompT genes were detected by PCR. Antibacterial and antibiofilm activities of probiotic CFS were assessed using agar well diffusion and microtiter inhibition assays. Statistical analysis included χ^2 *testing with significance at* $p \le 0.05$.

Results. Sixty culture samples had E. coli (60%). E. coli O157:H7 isolates comprised 11.7% ($p < 0.001$)*. High resistance rates were seen for nalidixic acid (88.6%), ceftazidime (86.6%), cefepime (76.6%), cefotaxime (70%), and aztreonam (73.3%). 55% of isolates had XDR traits and 45% had MDR traits* ($p > 0.05$)*. All MDR isolates had fimH and ompT virulence genes, with 59% forming moderate biofilms* ($p < 0.05$)*. Antibacterial activity of probiotic CFS was concentration-dependent, with maximum inhibition zones at 75 and 100 μg/mL. L. reuteri inhibited E. coli O157:H7 more than L. plantarum* ($p < 0.05$)*, although L. plantarum had stronger antibiofilm effects against robust biofilm-forming isolates* ($p < 0.05$)*.*

Conclusions. Uropathogenic E. coli provides a treatment challenge because to its multidrug-resistant and extensively drug-resistant phenotypes, biofilm formation, and universal carriage of fimH and ompT in pediatric UTI isolates. Probiotic metabolites, especially L. reuteri against E. coli O157:H7 and L. plantarum against biofilm-producing E. coli, exhibit high antibacterial and antibiofilm activities and can improve treatment outcomes and reduce recurrence in multidrug-resistant pediatric UTIs.

Keywords: *Escherichia coli, urinary tract infection, biofilm, Lactobacillus reuteri, Lactobacillus plantarum, fimH, ompT.*

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Пробіотичне інгібування уропатогенних *Escherichia coli* у дітей з інфекціями сечової системи: антибіоплівкові та антимікробні ефекти *Lactobacillus reuteri* та *Lactobacillus plantarum*

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Резюме. *Escherichia coli є провідним збудником інфекцій сечової системи (ІСС), однак зростання антимікробної резистентності, наявність генів вірулентності та формування біоплівок ускладнюють успішність лікування. Метаболіти, отримані з пробіотиків, розглядаються як потенційна додаткова стратегія для зменшення персистенції мультирезистентних (MDR) уропатогенів; проте дані щодо ІСС у дітей залишаються обмеженими. Метою роботи було визначити профіль антимікробної резистентності, здатність до формування біоплівок та поширеність генів вірулентності серед ізолятів E. coli від дітей з ІСС, а також оцінити антибактеріальну й антибіоплівкову активність безклітинних супернатантів (CFS) Lactobacillus plantarum і Lactobacillus reuteri.*

Методи. Проаналізовано 100 зразків сечі від дітей віком від 1 місяця до 10 років. Ідентифікацію бактерій виконували за допомогою культуральног дослідження, біохімічного профілювання та VITEK. Антибіотикочутливість визначали методом Кірбі-Бауера. Формування біоплівок кількісно оцінювали за допомогою тесту з кристалічним фіолетовим, а гени fimH та ompT виявляли методом ПЛР. Антибактеріальну та антибіоплівкову активність пробіотичних CFS оцінювали методом дифузії в агарі та тестами інгібування у мікропланшетах. Статистичний аналіз включав χ^2*-тест із рівнем значущості* $p \leq 0.05$*.*

Результати. Культурально позитивними були 60 зразків (60%) сечі, усі ідентифіковані як E. coli. E. coli O157:H7 становила 11,7 % ізолятів ($p < 0.001$)*. Висока резистентність відзначена до налідиксової кислоти (88,6%), цефтазидиму (86,6%), цефепіму (76,6%), цефотаксиму (70%) та азтреонаму (73,3%). Фенотипи MDR та XDR виявлені у 45% та 55% ізолятів відповідно* ($p > 0.05$)*. Переважало помірне формування біоплівок (59%;* $p < 0.05$*), а гени вірулентності fimH і ompT були присутні у 100% MDR-ізолятів. Пробіотичні CFS проявили концентраційно залежну антибактеріальну активність, із максимальними зонами інгібування за 75 і 100 μg/mL. L. reuteri продемонструвала значно сильнішу інгібіцію E. coli O157:H7 порівняно з L. plantarum* ($p < 0.05$)*, тоді як L. plantarum виявила значно потужніший антибіоплівковий ефект проти біоплівкоутворюючих ізолятів E. coli* ($p < 0.05$)*.*

Висновки. Ізоляти E. coli у дітей з ІСС характеризуються поєднанням MDR/XDR-фенотипів, здатністю до утворення біоплівок і універсальною наявністю fimH та ompT, що підкреслює складність лікування уропатогенних E. coli. Виражені антибактеріальні та антибіоплівкові властивості метаболітів пробіотиків, зокрема L. reuteri проти E. coli O157:H7 та L. plantarum проти біоплівкоутворюючих ізолятів E. coli, підтверджують їх потенціал як допоміжних засобів для покращення результатів лікування та зниження рецидивів MDR-асоційованих педіатричних ІСС.

Ключові слова: *Escherichia coli, O157:H7, діти, інфекція сечової системи, біоплівка, антибіотикорезистентність, Lactobacillus plantarum, Lactobacillus reuteri, пробіотики, fimH, ompT.*

Introduction. One major public health concern around the world is the prevalence of urinary tract infections (UTIs), which are a leading cause of bacterial infections in children [1, 2]. Clinically relevant are their long-term effects, such as pyelonephritis, renal scarring, hypertension, and chronic kidney disease in old age, and high recurrence rate. Recent epidemiological studies show that UTIs are exceedingly widespread across all age groups internationally, yet diagnostic uncertainty and postponed treatment keep them a burden for healthcare systems [1, 3]. Special consideration must be given to pediatric UTIs due to the substantial correlation between infections in early life and kidney morbidity [2, 4]. About three quarters to three quarters of all UTIs in children are caused by uropathogenic *E. coli* (UPEC), which has strong pathogenic mechanisms such immune-evasive outer membrane proteases and fibrial adherence to uroepithelial cells [5]. Persistent and recurring infections caused by UPEC can be attributed to certain virulence

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markers, including as fimH and ompT, which facilitate robust epithelial binding and resistance to host defenses [6, 7]. The fact that E. coli O157:H7 has been linked to serious urinary tract infections and systemic problems in kids is another cause for worry [8, 9].

UTI care is more complicated due to antibiotic resistance. MDR and XDR pediatric UPEC strains are becoming more common, restricting treatment options and weakening empirical therapy [10, 11]. There is a critical clinical need for complementary or alternative therapy approaches since antibiotic overuse leads to dysbiosis and raises the likelihood of recurring UTIs [12]. The development of biofilms affects both the persistence of UPEC and the therapeutic failure. Recurrence can occur even when a patient appears to be responding well clinically, because bacteria embedded in biofilms are far more resistant to medications [13, 14]. Therefore, to protect kidney health and reduce the chance of recurrence, it may be prudent to employ treatments that restrict bacterial growth and biofilm development. An appealing alternative to antibiotics for treating UTIs, probiotics compete with pathogens for epithelial adhesion sites, produce antimicrobial metabolites, suppress biofilms, and enhance mucosal immunity [15, 16]. Evidence suggests that certain *Lactobacillus* species may reduce UTI recurrence and act synergistically with conventional therapy [17, 18]. However, the inhibitory ability of locally isolated probiotic strains against pediatric UPEC, particularly MDR isolates and *E. coli* O157:H7, remains inadequately explored, and few studies have evaluated the interaction between probiotic metabolites and bacterial virulence determinants such as fimH and ompT [19].

The present study investigates the antibacterial and antibiofilm effects of two locally isolated probiotics, *Lactobacillus reuteri* and *Lactobacillus plantarum*, against clinical UPEC strains obtained from pediatric patients with UTIs, including *E. coli* O157:H7. The study also evaluates the prevalence of the fimH and ompT virulence genes and examines their association with probiotic susceptibility.

Materials and methods. *Study design and sample collection*. A cross-sectional laboratory investigation was conducted between March and August 2024. Ethical approval was obtained from the College of Science, University of Diyala (Approval No. 2024AEBT143). Parental or legal guardian informed consent was obtained prior to sample collection. Patient confidentiality was maintained by anonymizing data throughout the study.

Pediatric patients at Baqubah Teaching Hospital and Al-Batool Teaching Hospital in Diyala Province, Iraq, were clinically diagnosed with UTIs. The patients ranged in age from one month to ten years old, and 100 urine samples were obtained from them. For children who have completed potty training, we used mid-stream clean-catch urine, whereas for babies and toddlers, we used sterile pee bags as needed. In less than an hour after collection, every single sample had made its way to the microbiological lab for analysis.

Bacterial isolation and identification. Urine samples were streaked on MacConkey agar for the primary isolation of Gram-negative bacteria. Suspected Escherichia coli colonies were purified and sub-cultured on Sorbitol MacConkey agar (SMAC) supplemented with cefixime (50 μg/L) and potassium tellurite (2.5 mg/L) for selective isolation of *E. coli* O157:H7. Plates were incubated aerobically at 42 °C for 24 hours as previously described by Bedada et al. [20].

Biochemical identification was performed using standard diagnostic tests, including oxidase, catalase, indole production, methyl red, Voges–Proskauer, citrate utilization, urease, sugar fermentation, motility, and decarboxylation of ornithine and lysine [21]. Hemolysis type was assessed on 5% sheep blood agar following 24 hours of incubation at 37 °C. Final confirmation was achieved using the VITEK Compact 2 System.

Biofilm formation detection. Biofilm formation ability of *E. coli* isolates was assessed using the qualitative tube method. Briefly, isolates were cultured in tryptic soy broth supplemented with 1% glucose and incubated at 37 °C for 24 hours. Tubes were washed with distilled water, air-dried, stained with 0.5% crystal violet for 10–15 minutes, rinsed, and allowed to dry. A visible violet layer on the inner wall indicated positive biofilm formation [13].

Antibiotic susceptibility testing. Antimicrobial susceptibility was determined for 60 *E. coli* isolates using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Sixteen commercial antibiotic disks (Himedia, India) were tested: meropenem (10 μg), piperacillin (100 μg), cefotaxime (30 μg), levofloxacin (5 μg), ceftazidime (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), cefoxitin (30 μg), imipenem (10 μg), cefepime (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), augmentin (30 μg), aztreonam (30 μg), piperacillin–tazobactam (30 μg), and ceftriaxone (30 μg). After 24 hours of incubation at 37 °C, inhibition zone diameters were measured and interpreted in accordance with CLSI standards [22, 23].

Molecular detection of virulence genes. *DNA extraction*. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instructions. DNA purity and concentration were checked using a Nanodrop spectrophotometer and stored at –20 °C.

PCR amplification of fimH and ompT. Conventional PCR was performed to detect fimH and ompT genes using gene-specific primers obtained from Macrogen. Primer sequences, annealing temperatures, and expected product sizes are shown in Table 1.

Table 1

Primer sequences, annealing temperatures, and expected PCR product sizes used to detect fimH and ompT genes in *E. coli* isolates

Primer Name	Sequence	Annealing Temp. (°C)	Product size (bp)	Reference
ompT-F *ompT-R*	5'-ATCTAGCCGAAGAAGGAGGC-3' 5'-CCCGGGTCATAGTGTTCATC-3'	60	559	[23]
fimH-F *fimH-R*	5'-AACAGCGATGATTTCCAGTTTGTGTG-3' 5'-TTGCGTACCAGCATTAGCAATGTCC-3'	60	465	[24]

Reactions were prepared in 20 µL volumes containing 3 µL DNA template and 17 µL of primer working solution on ice. PCR cycling was performed according to standardized thermal profiles for each primer set [24, 25].

Gel electrophoresis. PCR products were separated on 1.5% agarose gel in 1× TBE buffer. Gels were stained with ethidium bromide, run at 5 V/cm^2 for 55 minutes, and visualized under UV transillumination. A 100 bp DNA ladder served as a size marker.

Source and preparation of probiotic isolates. Locally isolated *Lactobacillus reuteri* and *Lactobacillus plantarum* strains were obtained from the Department of Agriculture, University of Salah Al-Din. Identification was confirmed by colony morphology, Gram staining, catalase/oxidase testing, carbohydrate fermentation, growth at 15 °C and 45 °C, and tolerance to bile salts and acidic pH [26].

Antibacterial activity of probiotic cell-free supernatants. Antimicrobial activity was evaluated using the agar well diffusion assay. *E. coli* and *E. coli* O157:H7 lawn cultures (10^5–10^7 CFU/mL) were prepared on Mueller-Hinton agar. Cell-free supernatants (CFS) were obtained by centrifuging probiotic cultures at 6000×g for 15 minutes and filtering through 0.22 µm membranes. Wells (5 mm) were filled with 100 µL of CFS and plates were pre-cooled for 2 hours before incubation at 37 °C for 24 hours. Inhibition zones were measured in millimeters [27].

Antibiofilm activity of probiotic cell-free supernatants. Antibiofilm activity was assessed using a modified crystal violet microtiter assay [28]. After co-incubation of bacterial suspensions with 100 µL CFS in 96-well plates for 24 hours at 37 °C, wells were washed with sterile water, fixed with methanol, stained with 0.1% crystal violet, and eluted with ethanol. Absorbance was determined at 570 nm using an ELISA reader. Percentage biofilm inhibition was calculated relative to untreated controls. All experiments were performed in triplicate.

Statistical analysis. Clinical and demographic data were reported as percentages and frequencies. The Pearson Chi-square test was used to compare groups. P-values below 0.05 indicated statistical significance. We ran statistical studies in SPSS 22.0 and Excel 2013.

Results. One hundred samples were taken, with 65 from women and 35 from men. Patients reported 62% fever, 71% flank pain, and 58% dysuria. 35% had cystitis, 68% had pyelonephritis.

Culture characteristics and distribution of E. coli isolates. Sixty percent of the urine samples showed bacterial development, while forty percent did not. Each culture-positive sample was positively identified as belonging to E. coli by morphology of the colonies, microscopic evaluation, biochemical testing, and VITEK confirmation. The percentage of E. coli positivity was lower in males (54.28%) compared to females (61.07%) ($p < 0.05$). Using Sorbitol MacConkey agar, seven out of sixty E. coli isolates (11.66% of the total) were determined to be O157:H7 strains, while fifty-three (88.34%) were determined to be non-O157 strains ($p < 0.001$). Isolation rates of E. coli were highest in children ages 1-2 (30%), then in those aged 3-4 (18.33%), and finally in those aged 9-10 (18.33%). The lowest proportions occurred in infants <1 year (11.66%) and children aged 7–8 years (8.33%), with statistically significant differences across age groups ($p < 0.01$).

Antimicrobial resistance profile. Antibiotic susceptibility testing demonstrated widespread resistance among the 60 isolates (Fig. 1).

The highest resistance rates were observed for nalidixic acid (88.6%), ceftazidime (86.6%), cefepime (76.6%), aztreonam (73.3%), and cefotaxime (70%). Carbapenem resistance was recorded in 20% of isolates for meropenem and 30% for imipenem. Based on resistance phenotypes, 27 isolates (45%) were classified as multidrug-resistant (MDR) and 33 isolates (55%) as extensively drug-resistant (XDR), with no significant difference between groups ($p > 0.05$).

Biofilm formation capacity. Most isolates formed moderate biofilm (59%), followed by strong (22%) and weak (19%) biofilm production categories ($p < 0.05$) (Fig. 2). Six strong biofilm-producing isolates were selected for subsequent antibiofilm testing.

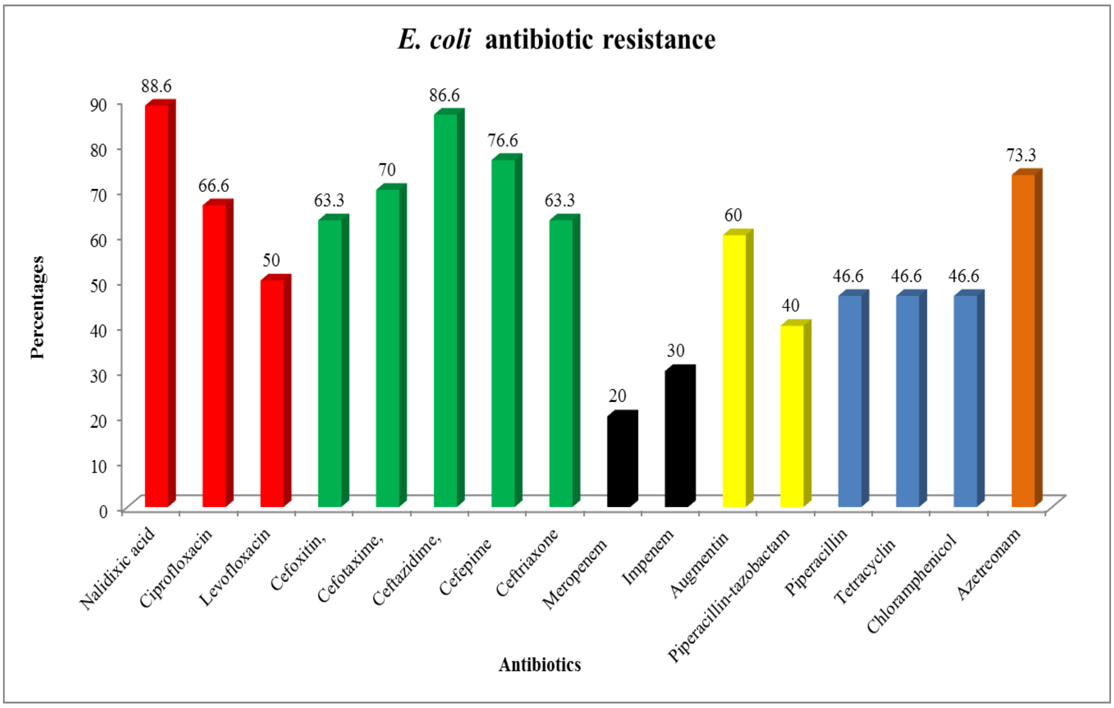


Fig.1. Antibiotic resistance profile of *E. coli* isolates.

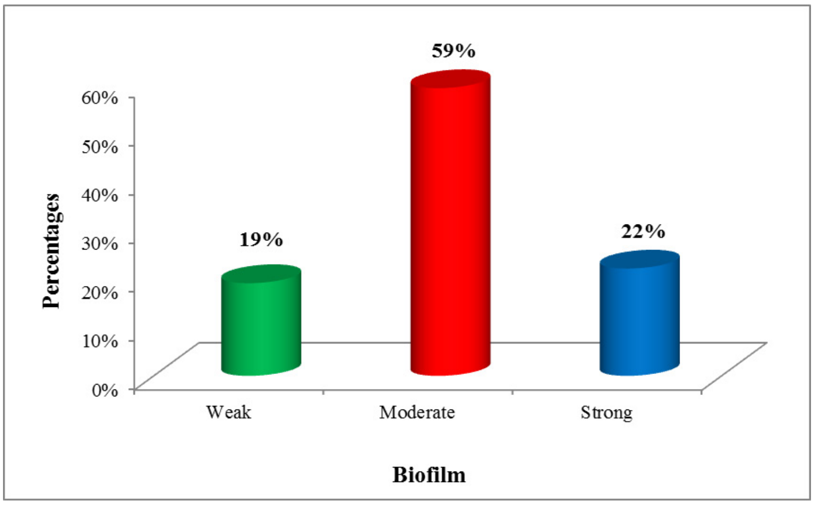


Fig. 2. Biofilm formation capacity of *E. coli* isolates.

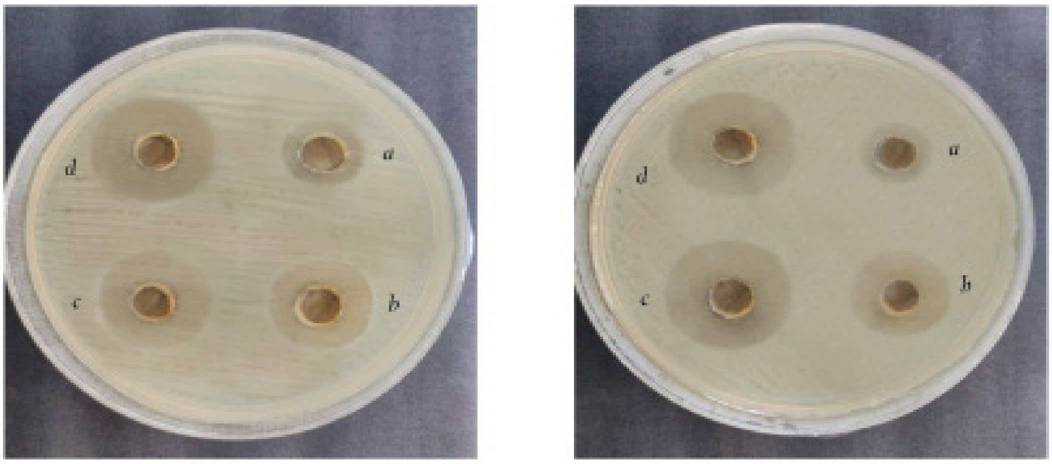
Detection of fimH and ompT genes. Multiplex PCR analysis of the 27 MDR *E. coli* isolates demonstrated that both fimH (465 bp) and ompT (559 bp) genes were present in all isolates (100%). The universal co-occurrence of these adhesion- and immune-evasion–associated genes among MDR isolates suggests a strong virulence profile across the population.

Antibacterial activity of probiotic cell-free supernatants. Both *Lactobacillus reuteri* and *Lactobacillus plantarum* cell-free supernatants exhibited inhibitory effects against *E. coli* and *E. coli* O157:H7. At 75 and 100 µg/mL, *L. reuteri* produced the largest inhibition zones against *E. coli* (22 mm and 26 mm) and *E. coli* O157:H7 (27 mm), showing significant differences compared to *L. plantarum* ($p < 0.05$) (Table 2; Figures 3 and 4).

Table 2

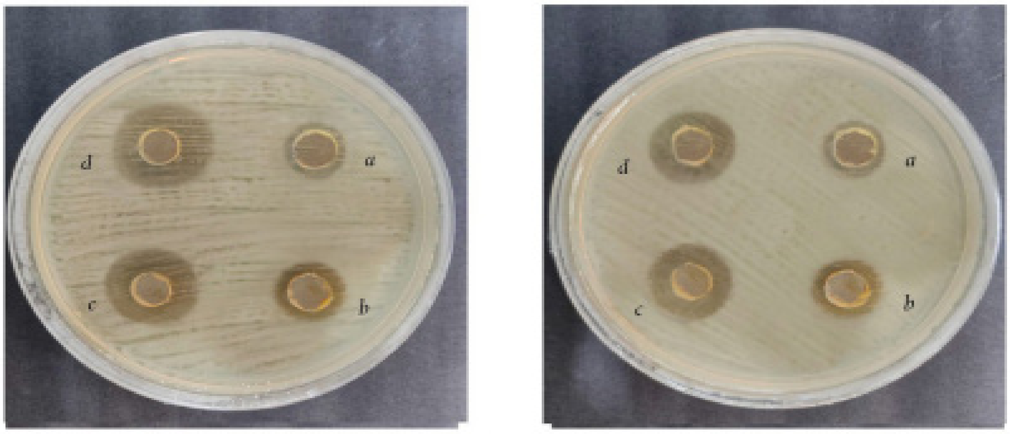
Antibacterial activity of *Lactobacillus reuteri* and *Lactobacillus plantarum* cell-free supernatants (CFS) against *E. coli* and *E. coli* O157:H7 expressed as zone of inhibition

Isolated bacteria	CFS concentration (μg/mL)	*L. reuteri* (mm)	*L. plantarum* (mm)	p-value
E. coli	25	16	12	$p > 0.05$
	50	19	15	$p > 0.05$
	75	22	20	$p > 0.05$
	100	26	22	$p > 0.05$
Overall p-value (*E. coli*)	–	$p < 0.05$	$p < 0.05$	–
E. coli O157:H7	25	15	12	$p > 0.05$
	50	20	14	$p < 0.05^*$
	75	27	18	$p < 0.05^*$
	100	27	18	$p < 0.05^*$
Overall p-value (*E. coli* 157:H7)	–	$p < 0.05$	$p < 0.05$	–



E. coli *E. coli* O157:H7

Fig. 3. Antibacterial activity of *Lactobacillus reuteri* (CFS) against *E. coli* and *E. coli* O157:H7.
Zone of inhibition (mm) measured by agar well diffusion at CFS concentrations of 25, 50, 75, and 100 μg/mL.
Letters (a, b, c, d) correspond to 25, 50, 75, and 100 μg/mL of CFS, respectively.



E. coli *E. coli* O157:H7

Fig. 4. Antibacterial activity of *Lactobacillus plantarum* (CFS) against *E. coli* and *E. coli* O157:H7.
Zone of inhibition (mm) measured by agar well diffusion at CFS concentrations of 25, 50, 75, and 100 μg/mL.
Letters (a, b, c, d) correspond to 25, 50, 75, and 100 μg/mL of CFS, respectively.

Antibiofilm effects of probiotic cell-free supernatants. Antibiofilm activity was evaluated using six *E. coli* isolates previously characterized as strong biofilm producers. Both *Lactobacillus reuteri* and *Lactobacillus plantarum* cell-free supernatants demonstrated inhibitory effects against biofilm formation, although the magnitude of inhibition varied across isolates (Table 3).

Table 3

Antibiofilm activity of *Lactobacillus plantarum* and *Lactobacillus reuteri* cell-free supernatants against strong biofilm-forming *E. coli* and *E. coli* O157:H7 isolates

Isolate	*L. plantarum* (inhibition %)	*L. reuteri* (inhibition %)	p-value
E1 (*E. coli*)	37	39	p > 0.05
E2 (*E. coli*)	53	61	p > 0.05
E3 (*E. coli*)	56	45	p > 0.05
E04 (*E. coli* O157:H7)	39	50	p > 0.05
E05 (*E. coli* O157:H7)	43	46	p > 0.05
E06 (*E. coli* O157:H7)	30	41	p > 0.05
Overall p-value	p < 0.05*	p > 0.05	–

L. plantarum showed the greatest antibiofilm activity against isolates E2 and E3 (*E. coli*), with inhibition rates of 53% and 56%, respectively, while the lowest activity was observed against isolate EO6 (*E. coli* O157:H7), with 30% inhibition ($p < 0.05$).

In contrast, *L. reuteri* exhibited relatively uniform inhibition across isolates, and no statistically significant differences were detected between inhibition levels ($p > 0.05$). When the two probiotic species were compared overall, *L. plantarum* displayed significantly higher antibiofilm activity than *L. reuteri* against the group of strong biofilm-producing isolates ($p < 0.05$), although the difference was not evident on an isolate-by-isolate basis.

Discussion. Although *E. coli* is consistently recognized as the leading cause of pediatric urinary tract infections, there remains a lack of studies that simultaneously evaluate antimicrobial resistance, biofilm formation, virulence gene carriage, and the inhibitory potential of probiotic metabolites against clinical isolates. In regions where antibiotic resistance is rapidly increasing and monitoring is lacking, this difference stands up even more. By integrating clinical microbiology with probiotic inhibitory assays, this study addresses a gap in the literature and provides novel insights.

Some reports were noted that E. coli is still prevalent in juvenile UTIs [**1, 38**], But, Lee [**3**] brought attention to the challenges of distinguishing between asymptomatic bacteriuria and actual infection. Our findings support these tendencies and add to the increasing amount of data connecting the prevalence of the clinically concerning serotype *E. coli* O157:H7 to infections of the intestines and hemolytic uremic syndrome in children. The challenges in managing recurrent UTIs were emphasized by [**12**], while the increasing menace of resistant UPEC strains renders the new dataset therapeutically significant [**5, 10**].

It reflects global antibiotic resistance tendencies. Miller [**39**] discovered that resistant strains boost UTI complications in susceptible patients. The growing resistance in pediatric isolates were demonstrated [**2, 4**], supporting our findings. Middelkoop [**40**] confirmed the emergency diagnostic value of fast microbiological tools. Kherroubi [**41**], who identified GyrA and ParC mutations as fluoroquinolone resistance pathways, matches our isolates' patterns. Tellapragada [**42**] and Al-Hasnawy [**43**] discovered widespread cephalosporin and aztreonam resistance.

Ramírez Castillo [**14**], Qahtan [44], and Musafer [**45**] found that biofilms contribute to recurrent UTIs and treatment failure, and a significant percentage of isolates produce biofilms. All MRSA strains have fimH and ompT genes, supporting Desloges [**6**] and Zhang [**7**]'s conclusions that they aid adherence, antimicrobial peptide resistance, and colonization.

Antibiotics are becoming less effective, so research into probiotic-based alternatives or supplements has increased. Latif [**15**] and Qasemi [**16**] stressed probiotics' benefits for bacterial infections, but Akgül [**17**] demonstrated their UTI prevention potential. Luo [**46**] and Park [**47**] investigated Lactobacillus strains' immune-modulatory effects, and Soltani [**19**] found that their metabolites diminish pathogenic *E. coli*. Carvalho [**48**] found that lactobacilli inhibit urinary tract device biofilms. Recent studies have found antibacterial and antibiofilm effects in probiotic metabolites [**49**].

Our finding that both *L. plantarum* and *L. reuteri* cell-free supernatants inhibited this supports previous findings that *E. coli* and *E. coli* O157:H7 are probiotic-resistant in wastewater systems [**20, 21**], food models like white-brined cheese and minced beef [**50, 51**], and biological systems like the intestines, rumen, wounds, and infections [**52-57**]. Probiotic metabolites decrease bacterial virulence, adhesion, and colonization [**18,**

58], supporting L. plantarum's better antibiofilm efficacy in this study.

Despite the fact that this study offers valuable therapeutic and microbiological insights, it is crucial to note that there are a number of limitations. To start, the results might not apply to other pediatric groups as the sample was just from one area. And secondly, because the molecular research only looked at the fimH and ompT genes, other resistance and virulence factors might have gone unnoticed. Thirdly, the study did not evaluate in vivo effects, host immunological interactions, or optimal dosing settings; it only examined the antibacterial and antibiofilm efficacy of probiotic cell-free supernatants in vitro. Fourth, recurrence rates after probiotic treatment were not assessed by long-term clinical follow-up in this investigation. Lastly, it would have been beneficial to study the effects of mixing probiotics with conventional antibiotics. This knowledge could be useful for real-world clinical implementation. In future studies with a clinical focus, that are multicenter and longitudinal, these limitations should be addressed.

Conclusions. Even though *E. coli* O157:H7 only accounts for a small fraction of UTIs in kids, our research confirms that it is the leading cause. The significant influence of the region's frequent empirical antibiotic use is demonstrated by the isolates' impressive resistance to numerous antibiotics.

Cell-free supernatants of *Lactobacillus reuteri* and *Lactobacillus plantarum* demonstrated concentration-dependent antibacterial activity against both *E. coli* and *E. coli* O157:H7. Although both probiotic strains showed comparable inhibitory effects against *E. coli*, *L. reuteri* produced stronger inhibition against *E. coli* O157:H7, whereas *L. plantarum* displayed superior antibiofilm activity against strong biofilm-forming *E. coli* isolates.

Overall, these findings indicate that probiotic metabolites possess promising antibacterial and antibiofilm properties against multidrug-resistant UPEC isolates. While *L. reuteri* appears more suitable for limiting the growth of *E. coli* O157:H7, *L. plantarum* may serve as an effective biofilm-targeting agent against *E. coli.* Probiotic-based strategies could therefore be considered as complementary approaches alongside antibiotics to help reduce treatment failure and recurrence in pediatric UTIs.

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