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Study of The Synergistic Activity of Grape Extract and Lactic Acid Bacteria Against Some Virulence Factors of Different Gram-Negative Bacteria

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Abstract

Background: Controlling infections is challenging due to the emergence of multidrug-resistant bacteria. plant extract Resveratrol and lactic acid bacteria metabolites have antimicrobial activity against Gram-negative bacteria, through mechanisms including the inactivation of virulence factors, and affecting membrane permeability and biofilm formation. This study aimed to investigate the effects of resveratrol and cell-free fluid (CFS) of the probiotic *Lactobacillus plantarum* against the phenotypic characteristics and virulence factors of Gram-negative bacteria isolated from urinary tract infections.

Methods: We analyzed 212 urine specimens from patients with urinary tract infections and identified bacterial isolates using phenotyping methods and the automated VITEK-2 system. Antibiotic susceptibility testing was performed using the disc diffusion method, while the inhibitory effects of CFS and natural plant extracts were assessed using the agar well diffusion test. The minimum inhibitory concentration (MIC) was estimated using the microdilution method. Finally, the interaction between resveratrol and cell-free cellular extract (CFS) was investigated against several phenotypic traits and virulence factors in Gram-negative isolates.

Results: Gram-negative bacterial strains isolated from urinary tract infections were found to be resistant to several drugs. Resveratrol and CFS exhibited a significant bactericidal effect on the growth of Gram-negative isolates, with the minimum inhibitory concentration (MIC) being 1000 µg/ml for resveratrol,

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and 40 mg/ml for CFS. Sub inhibitory concentrations showed a clear effect on several studied traits, including reduced biofilm formation, capsule production, swarming motility, and the restoration of susceptibility to the antibiotics used in the study.

conclusion: The use of natural agents such as resveratrol and lactic acid bacteria products can

effectively modify the phenotypic traits and virulence factors of gram-negative bacteria, supporting their potential role as complementary or alternative strategies in the treatment for the causes of infection.

دراسة التأثير التآزري لمستخلص العنب وبكتيريا حمض اللاكتيك في تثبيط بعض عوامل الضراوة لدى بكتيريا سالبة الغرام مختلفة الأنواع.

جمال عامر اللهيبي وليث مصلح نجيب

الخلاصة

المقدمة: يُعدّ التحكم في العدوى تحديًا نظرًا لظهور البكتيريا المقاومة للأدوية المتعددة. يمتلك المستخلص النباتي الريسفيراترول ومستقلبات بكتيريا حمض اللاكتيك نشاطًا مضادًا للميكروبات ضد البكتيريا سالبة الغرام، وذلك من خلال آليات تشمل تعطيل عوامل الضراوة، والتأثير على نفاذية الغشاء وتكوين الأغشية الحيوية.

الهدف من الدراسة: هدفت هذه الدراسة إلى التحقق من تأثيرات الريسفيراترول والسائل الخالي من الخلايا (CFS) لبكتيريا البروبيوتيك Lactobacillus plantarum على الخصائص الظاهرية وعوامل الضراوة للبكتيريا سالبة الغرام المعزولة من التهابات المسالك البولية.

طريقة العمل: قمنا بتحليل 212 عينة بول من مرضى مصابين بالتهابات المسالك البولية، وحددنا العزلات البكتيرية باستخدام طرق التنميط الظاهري ونظام VITEK-2 الآلي. أُجري اختبار حساسية المضادات الحيوية باستخدام طريقة انتشار القرص، بينما قُيّمت التأثيرات المثبطة للسائل الخالي من الخلايا والمستخلصات النباتية الطبيعية باستخدام اختبار انتشار الآبار في وسط الآجار. تم تقدير الحد الأدنى للتركيز المثبط (MIC) باستخدام طريقة التخفيف الدقيق. وأخيرًا، دُرست فعالية التفاعل بين الريسفيراترول والمستخلص الخلوي الخالي من الخلايا (CFS) في التأثير على العديد من الصفات المظهرية وعوامل الضراوة في عزلات البكتيريا سالبة الغرام.

النتائج: تبين أن سلالات البكتيريا سالبة الغرام المعزولة من التهابات المسالك البولية مقاومة للعديد من الأدوية. أظهر الريسفيراترول والمستخلص الخلوي الخالي من الخلايا تأثيرًا قاتلًا للبكتيريا على نمو عزلات البكتيريا سالبة الغرام، حيث بلغ الحد الأدنى للتركيز المثبط 1000 (MIC) ميكروغرام/مل للريسفيراترول، و40 ملليغرام/مل للمستخلص الخلوي الخالي من الخلايا. وأظهرت التراكيز دون المثبطة تأثيرًا واضحًا على العديد من الصفات المدروسة، بما في ذلك انخفاض تكوين الأغشية الحيوية، وإنتاج الكبسولات، والحركة الجماعية، واستعادة حساسية البكتيريا للمضادات الحيوية المستخدمة في الدراسة.

الاستنتاج : يمكن أن يؤدي استخدام عوامل طبيعية مثل الريسفيراترول ومنتجات بكتيريا حمض اللاكتيك إلى تعديل فعال للسمات الظاهرية وعوامل ضراوة البكتيريا سالبة الجرام، مما يدعم دورها المحتمل كاستراتيجيات تكميلية أو بديلة في علاج أسباب العدوى.

1.Introduction

Bacteria are the primary cause of various infections, especially urinary tract infections. It is considered the primary cause of infection for millions of people and therefore represents a significant health and economic burden..(He *et al.*, 2025). Gram-negative bacteria such as *Escherichia coli*(34.7%), *Klebsiella pneumoniae*(23.1%), *Proteus mirapilis*(11.5%), and *Pseudomonas aeruginosa*(17.3%) are the most frequent causative agents of these infections, whether acquired in the community or in hospitals (Adekanmbi *et al.*, 2022). The increasing prevalence of multidrug-resistant (MDR) strains has made it difficult to control these infections using conventional antibiotic therapies, necessitating the search for safer and more effective treatment alternatives(Parmanik *et al.*, 2022).The overuse of antibiotics has led to high levels of antibiotic resistance in clinically isolated bacteria, including bacterial urinary tract infections, particularly those caused by *E. coli* and *P. aeruginosa*(Al-jumaili and Ahmed, 2024). These studies have identified multidrug-resistant isolates, including those resistant to penicillin (100% to ticarcillin, 95% to amoxiclav, 92.5% to amoxicillin, and 95% to ampicillin), third-generation cephalosporins (95% to cefixime and 100% to ceftazidime), and aminoglycosides (85.3% to gentamicin and 76.2% to tobramycin. (Al-Zaidi, 2016) Trans-resveratrol (t-RSV), a naturally occurring polyphenolic compound found in grapes and peanuts, has garnered significant attention due to its potential health benefits, including its antioxidant, anti-aging, and antimicrobial properties(Bejenaru *et al.*, 2024). This compound exhibits antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria, as well as some viruses and fungi. It has recently gained popularity as a dietary supplement due to its good tolerability in humans (Abedini *et al.*, 2021). In addition, studies have shown that it inhibits Gram-negative bacteria, but at higher concentrations compared to Gram-positive pathogens (Mandal and Domb, 2024). Resveratrol also has antiviral activity against herpes simplex viruses, influenza viruses, respiratory syncytial virus, and Epstein-Barr virus(Coşkun *et al.*, 2025). In contrast, Lactic acid bacteria (LAB) are good probiotic organisms and one of the most commonly employed bacteria in the food business. It is used in the dairy industry and to improve food quality (Abedin *et al.*, 2024) . Some studies have also indicated that this bacteria has therapeutic effects in fighting specific infectious disorders, such as *Helicobacter pylori* (Saha and Saroj, 2022), and it has a function in regulating innate immunity and activating the immune response against pathogens (Moon *et al.*, 2022). Lactic acid bacteria (LAB) create bioactive chemicals such as organic acids, bacteriocins, hydrogen peroxide, and bioemulsifiers (Abdul Hakim, Xuan and Oslan, 2023). These natural compounds hinder the development of harmful microorganisms and biofilm formation by reducing pH, interrupting bacterial cell membrane formation, and altering quorum sensing (Aman *et al.*, 2021).Resveratrol has been demonstrated to have a synergistic impact with some antibiotics, enhancing their efficiency against multidrug-resistant Gram-negative isolates(Liu *et al.*, 2020). Resveratrol suppresses the production of virulence factors, compromises cell membrane integrity, and reduces bacterial adhesion and biofilm formation (Qin *et al.*, 2023). This synergistic impact provides a natural, safe, and ecologically friendly treatment option for antibiotic-resistant urinary tract infections (Xiao, Li and Sun, 2023).Therefore, this study aims to investigate the synergistic effect of resveratrol and natural products produced by Lactic acid bacteria (LAB) with some antibiotics on some phenotypic characteristics and expression of some virulence factors against a number of pathogenic bacteria.

2- Materials and Methods

2.1. Study Design and Inclusion Criteria

This study was conducted at Fallujah Teaching Hospital in Anbar, Iraq, from August 2025 to December 2025. Patients referred from the Urology Unit to the Central Laboratory Unit presented with burning and pain upon urination. They also reported discomfort or pain in the flanks. The study included 212 individuals over the age of 10 years. All research methods used in this study were approved by the Ethics Approval Committee of the University of Anbar in Ramadi, Iraq (Approval No. 845, August 2025). All participating patients provided written informed consent.

2.2. Collection of Samples

A total of (212) clinical specimens were collected from the urinary tract of patients visiting the laboratory unit at Fallujah Teaching Hospital during the period from 1 September to 30 November 2025. Information related to the patient, including age, sample collection date, residence, and other information, was recorded in a special form. The specimens were cultured on blood agar and MacConkey agar media using the schematic method. The dishes were incubated at 37°C for 24 hours aerobically (Karah *et al.*, 2020).

2.3. Bacterial Identification

Positive culture plates were used to identify Gram-negative isolates using standard microbiological techniques, including colony morphology, microscopy (Gram staining), and biochemical tests (Mahon and Manuselis, 2000). The VITEK2 Compact system (Biomerieux, USA) was used to confirm the identification of Gram-negative bacterial isolates. according to the manufacturer's instructions.

2.4. Inclusion and Exclusion Criteria

Only Gram-negative bacterial species that are among the most common causative agents of urinary tract infections were included in this study *E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa* .Among the collected isolates, only those showing high levels of resistance to multiple antibiotics were selected for further analysis.Isolates that exhibited susceptibility or low resistance to the tested antibiotics were excluded, as the aim of the study was to evaluate the synergistic effect of the tested agent against multidrug-resistant uropathies.

2.5. Antibiotic Susceptibility

The susceptibility of bacterial isolates to antibiotics was determined using the Kirby-Bauer disc diffusion method, and Mueller-Hinton agar medium was used according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI)(Weinstein, 2018). Bacterial isolates were classified as sensitive (S), moderately sensitive (I), and resistant (R) based on the diameter of the inhibition zones (mm) for each antibiotic. Resistant isolates were defined as those that showed inhibition zones less than the value defined by CLSI, while sensitive isolates showed inhibition zones equal to or greater than the recommended values Table1.

Table1: Antibiotic Susceptibility Rates of Gram-Negative Bacterial Isolates

Antibiotic	Concentration (µg/disc)	Sensitive	Intermediate	Resistant
Cefixímes	30	0(0%)	0(0%)	23(100%)
Cefotaxime	30	0(0%)	0(0%)	23(100%)
Ceftriaxone	30	0(0%)	1(4.34) %	22(95.65) %
Levofloxacin	5	1(4.34) %	0(0%)	22(95.65) %
Ciprofloxacin	5	4(17.39%)	1(4.34%)	18(78.26%)
Trimethoprim	25	4(17.39%)	1(4.34%)	18(78.26%)
Clotrimazole	25	5(21.73%)	0(0%)	18(78.26%)
Gentamicin	10	10(43.47%)	2(8.69%)	11(47.82%)
Cefepime	30	9(39,1%)	1(4.34%)	13(56.52%)
Nitrofurantoin	300	4(17.39%)	0(0%)	19(82.60%)
Ceftazidime	30	4(17.39%)	5(21.73%)	14(60.86%)
Amikacin	30	13(56.52%)	4(17.39%)	6(26%)
Meropenem	10	15(65.21%)	4(17.39%)	4(17.39%)
Colistin	10	0(0%)	18(78.26%)	5(21.73%)
Tigecycline	15	0(0%)	8(34.78%)	15(65.21%)

2.6. Preparation of Neutral Extract

2.6.1 Preparation of Resveratrol Solution

Pure resveratrol was purchased from Macclesfield-UK in the United Kingdom with a purity of 99.9%, and a resveratrol extract was prepared at a concentration of 200 mg/mL according to the method developed by (Paulo *et al.*, 2010).

2.6.2. Preparation of Lactobacillus Product

2.6.2. 1 Cultivation of Probiotic Bacteria

A commercial product containing various strains of *Lactobacillus acidophilus* (LAB) in capsule form was obtained. The solid contents of the capsule were rehydrated in a tube containing 5 mL of MRS broth, specifically formulated for culturing this strain. The medium was stirred with a sterile wooden stick and left to stand for 5 minutes. A portion of the bacterial suspension was then cultured onto MRS agar plates and incubated anaerobically at 37°C. After 48 hours of incubation, the resulting colonies were sub cultured to obtain pure cultures from the selected colonies(El Far *et al.*, 2024). Gram staining w performed on the isolated pure cultures. Biochemical tests were conducted to identify the isolated bacteria using *Lactobacillus* and related genera test kits for anaerobic bacteria. The diagnosis was confirmed using the Vitek II assay. The tests were performed according to the manufacturer's instructions (Macedo and Fredua-Agyeman, 2016).

2.6.2.2. Preparation of Lactobacillus Supernatant

A total of 200 mL of sterile MRS medium solution was inoculated with 0.5 McFarland's L. plantafom (1.5×10^6 CFU/mL) and incubated anaerobically for 48 h at 37°C. The culture broth was then centrifuged at 5000 rpm for 15 min, and the supernatant was collected. The supernatant was filtered using a Millipore filter (0.22 nm). After filtration, a ring filled with the filtered liquid was placed on an MRS agar plate and incubated for 48 h at 37°C under anaerobically controlled conditions to confirm the sterility of the filtered liquid. The filtered liquid was stored at 4°C until the next use (Gökmen *et al.*, 2024).

2.7. Antibacterial Activity of Natural Extracts

The agar well diffusion method was used to determine the antibacterial activity of resveratrol and the natural products of *Lactobacillus*, according to the method described by (Kiousi *et al.*, 2023) .Initially, a 100 µL sample of a 24-hour bacterial culture of four Gram-negative isolates in BHI broth was diffused onto a Mueller-Hinton agar surface. A 6 mm diameter well was then prepared for each extract using a sterile cork drill. Each well was filled with the crude extract liquid, while another well was filled with sterile distilled water as a negative control. The plates were then incubated at 37°C for 24 hours before measuring the diameter of the inhibition zone (in mm).

2.8. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined for resveratrol and the aqueous and alcoholic extracts of grape. Microdilution was performed in 96-well microplate dishes, using resazurin as an indicator of bacterial activity.A double dilution series was prepared, starting with the highest concentration in row A. Initial concentrations were 2000 µg/mL (2 mg/mL) for resveratrol and 200 mg/mL for both the aqueous and alcoholic extracts, with three replicates per extract to avoid error. 100 µL of Molar Hinton Bruth culture medium was added to each well, followed by 100 µL of each extract in row A. This was then transferred to row B, and so on, up to the final row H. A control was applied for each solvent individually, and positive and negative controls were used to monitor growth. Then, 10 µg of the standard bacterial suspension was added to the wells, and the plates were incubated at 37°C for 24 hours. Resazurin stain was then added, and the color change from blue-violet (indicating the absence of growth) to pink (indicating bacterial growth) was monitored to determine the minimum inhibitory concentration (MIC).

2.9. Effect of Resveratrol and Lactic Acid Products on the Morphological Characteristics of Gram-Negative Bacteria

Gram-negative bacteria were inoculated onto selective MacConkey agar. After confirming the purity of the culture, a single isolated colony was cultured in a bacterial suspension and then placed in distilled water at a specific concentration determined according to the McFarland standard. A specific amount of the standardized bacterial suspension was added to a broth of heart and brain extracts treated with 250 µg/ml resveratrol and lactic acid products at a concentration of 40 mg/ml, either individually or in a mixture of the two substances. After an incubation period, this mixture was re-cultured on different solid media to assess bacterial growth after exposure to the compounds. Finally, the phenotypic characteristics of the growing bacteria were studied to detect any phenotypic changes in response to the antimicrobial effects of resveratrol and lactic acid products. The effect of natural extracts on a range of phenotypic characteristics of bacterial isolates was evaluated using standard microbiological methods. Growth rate was determined by measuring optical density at 600 nm according to method (Ghaidaa, Yanchang and Hindi, 2014). Hemolysin production was assessed on blood agar(James and Cappuccino, 2017). Capsule production was assessed by observing the glossy appearance of bacterial colonies.(Chiarelli *et al.*, 2020). Oxidase activity was tested using standard oxidase reagents (Fadlallah, 2014). Lactose fermentation was examined on MacConkey agar(Maganga, 2019). Swarming motility was assessed on low-concentration blood agar (Ghaidaa, Yanchang and Hindi, 2014). Biofilm formation was assessed using the tube method with crystal violet staining (Tiwari *et al.*, 2017).

2.10. Phenotypic Modulation of Antibiotic Resistance

According to our modified method, which involved culturing Gram-negative bacterial isolates in heart and brain extract (BHI) broth, the density of the bacterial suspensions was adjusted to the McFarland standard of 0.5. Treatment groups were prepared using resveratrol at a concentration of 250 µg/ml under the influence of the inhibitor, lactic acid bacteria products at a concentration of 40 mg/ml, and a mixture of both. After incubation at 37°C for 24 hours, samples from each group were re-cultured on Mueller-Hinton agar. Antibiotic susceptibility testing was performed using the Kirby-Bauer diffusion method, and the plates were incubated for 24 hours at 37°C. The diameter of the inhibition zones was then measured and compared to the control group to evaluate the effect of the resveratrol and lactic acid bacteria treatments on bacterial susceptibility to antibiotics (Seukep *et al.*, 2016).

3. Results

3.1. Bacterial Isolates

A total of 212 urine specimens were collected from patients presenting at Fallujah Teaching Hospital. Of these, 92 samples (43.4%) were found to be positive based on culture and biochemical analysis. The remaining 120 samples (56.6%) tested negative. Among the positive specimens, 74 (80.5%) were from women and 18 (19.5%) were from men. The isolated bacteria were cultured as 23 Gram-negative (25%) and 69 Gram-positive (75%). Of the 92 patients presenting with urinary tract infections, 13 had *Escherichia coli,* 5 had *Klebsiella pneumoniae*, 2 had *Proteus mirabilis,* and 3 had *Pseudomonas aeruginosa.*

3.2. Antibiotics Susceptibility of Gram-Negative Bacteria:

The efficacy of fifteen antibiotics from different classes was evaluated against 23 strains of Gram-negative bacteria isolated from patients with urinary tract infections. The study used the disc diffusion method, in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI). The results indicated that the isolates exhibited varying levels of antibiotic resistance, as shown in Table 1. The isolates causing urinary tract infections showed significant resistance to different types of antibiotics. According to the multidrug resistance (MDR) definition, which requires

resistance to at least three antimicrobial drugs, *E. coli* (95%), *K. pneumoniae* (60%), *and P. mirabilis* and *P. aeruginosa* (100%) exhibited multidrug resistance Fig.1.

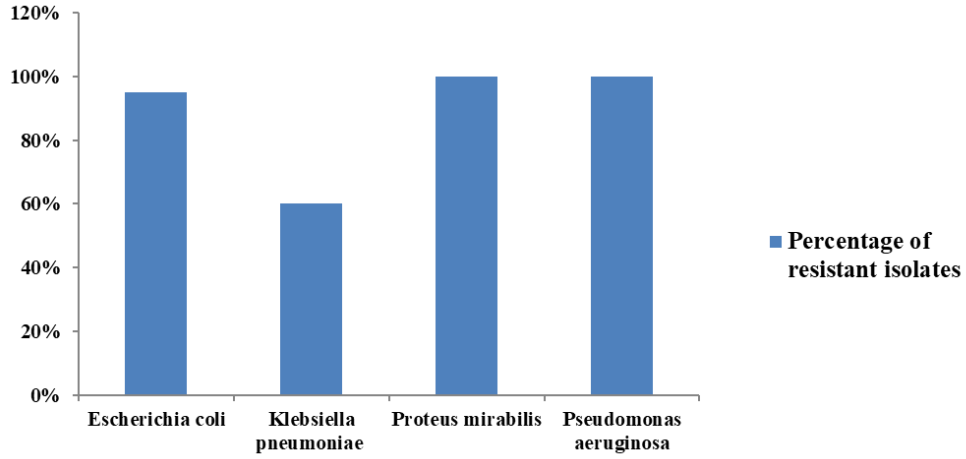

Figure1: Shows the Percentage of Multidrug-Resistant Bacterial Isolates.

3.3. Activity of Antibiotics with Resveratrol and Lactic Acid Bacterial Products in Zone Inhibition Tests

The results showed that resveratrol had an endogenous effect, with a concentration of 1000 mg/ml, it exhibited different effects on *E. coli* isolates, with an effect rate of 25% on the tested local isolates. As for its effect on *K. pneumoniae* isolates, the effect rate was 33.3% at the same concentration, with varying inhibition diameters as shown in Fig.2, *P. mirabilis* and *P. aeruginosa* were 100% resistant to resveratrol, showing no effect at any concentration.

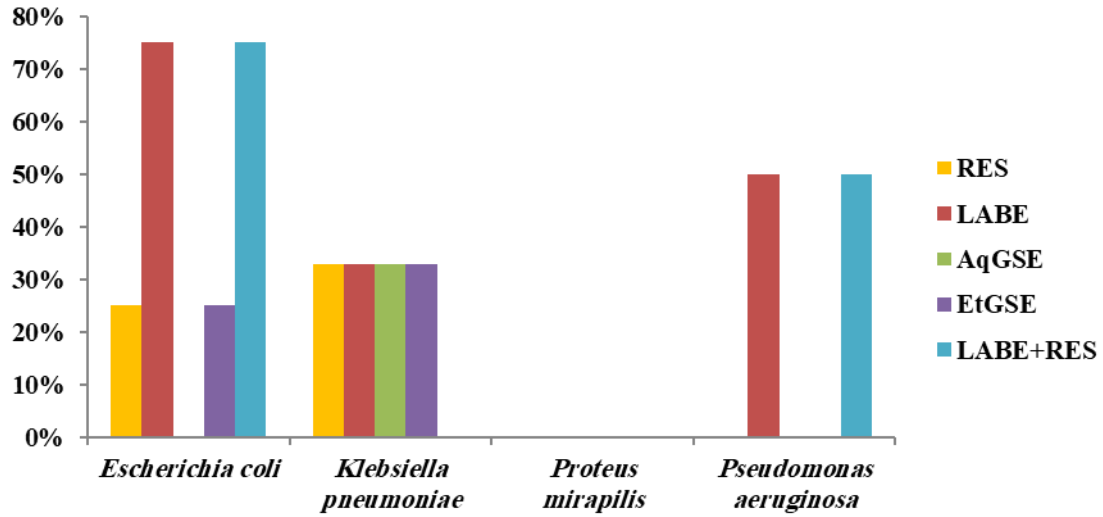

Figure2: Percentage Effect of Natural Extracts on Bacterial Isolates. Aqgse = Aqueous Grape Seed Extract. Etgse = Ethanolic Grape Seed Extract. RES =Resveratrol. AB= Antibiotic LABE =Lactic Acid Bacteria Extracts Lactic Acid Bacteria Extracts

The results showed that the filtrate produced by *Lactobacillus* spp. had a varying inhibitory effect on the tested bacterial isolates. It was 75% effective against local *E. coli* isolates, had no effect on *K. pneumoniae* and *P. mirabilis* isolates. 33.33% effective against *P. aeruginosa.*As for the aqueous extract of grape seeds, it showed no effect on *E. coli* ,*P. mirabilis* and *P. aeruginosa* isolates, while it showed a 33.33% effect on *K. pneumoniae.* The results also showed that the alcoholic extract of grape seeds also had a varying effect on the local bacterial isolates, as its effect on *E. coli* was 25%, on *K. pneumoniae* 33.33%, and it did not show any effect on *P. mirabilis* and *P. aeruginosa*. However, when using a mixture of resveratrol and *Lactobacillus* filtrate in 1:1 ratio, the results showed that this mixture had a 75% effect on *E. coli*, while *K. pneumoniae* and *P. mirabilis* had no effect. It had a 50% effect on *P. aeruginosa*, with varying and different inhibition diameters for all isolates.

3.4. Minimum Inhibitory Concentration (MIC)

The results showed that the resveratrol wells retained the blue color only up to row B, indicating a minimum inhibitory concentration of 1000 µg/ml against *E. coli* and *Klebsiella*, while it showed no effect on the other bacterial species used in the experiment (Cannatelli *et al.*, 2018). The aqueous and alcoholic extracts showed a color change only up to row A, indicating a minimum inhibitory concentration of 200 mg/ml. The bacterial filtrate from LAB bacteria exhibited varying inhibitory activity against different bacterial isolates at a concentration of 40 mg/ml.

3.5. Effect of Resveratrol and Lactic Acid Products on the Morphological Characteristics of Gram-Negative Bacteria

3.5.1. Growth Rate

After 24 hours of incubation, growth density was measured at a wavelength of 600 nm. All tubes showed growth, but at varying densities. The highest reading was observed in the tube containing only the bacteria. The lowest growth density was observed in the tubes treated with resveratrol. The tubes treated with LAB bacterial products showed less growth compared to the other treatments. The tube containing the mixture of resveratrol and LAB clear liquid showed moderate growth.

3.5.2. Hemolysin Production

The bacteria showed variation in hemolysin production, with *E. coli* producing more hemolysin than the other bacterial species used in the study. The results showed a significant and clear decrease in hemolysin-producing *E. coli* isolates treated with resveratrol. The effect of LAB bacterial products was less pronounced compared to resveratrol, while the mixture had a moderate effect on hemolysin production, but less than the effect of resveratrol alone, as shown in the Fig3.

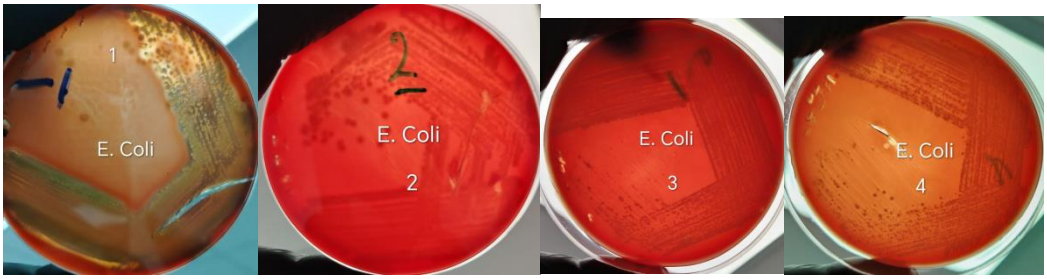

Figure3: Effect of Resveratrol, Lactic Acid Bacteria (LAB) Products, And Their Mixture on Hemolysin Production by Escherichia Coli Under Different Treatment Conditions. (1) untreated *E. coli*. **(2)** *E. coli* treated with resveratrol. **(3)** *E. coli* treated with lactic acid bacteria (LAB) products. **(4)** *E. coli* treated with a mixture of resveratrol and LAB products.

3.5.3. Capsule Production

The results showed that *K. pneumoniae* colonies were the most capsule-producing among the studied species. In the control group, these colonies exhibited a shiny, mucoid appearance, indicating high capsule production. In contrast, treatment of the bacterial isolates with resveratrol and lactic acid bacteria products, as well as the combined treatment of both, resulted in a significant loss of shine and a transformation of the colonies to a duller, darker, and less mucoid appearance compared to the control group. This treatment led to an almost complete loss of the shiny appearance associated with capsule production., as shown in the Fig.4.

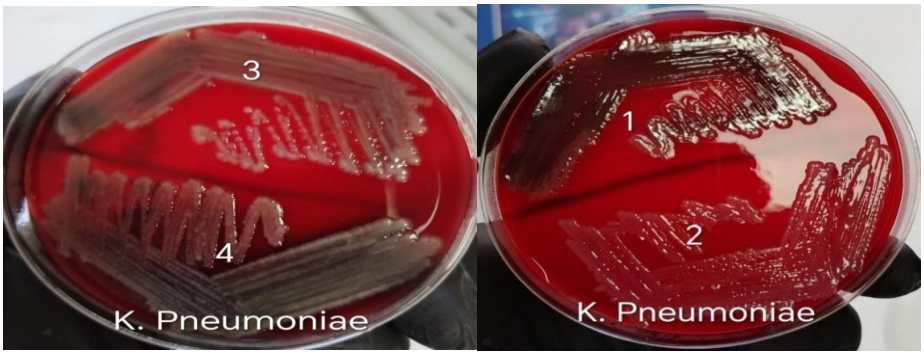

Figure4: In Vitro Evaluation of Capsule Production by Klebsiella Pneumoniae Under Different Treatment Conditions with Resveratrol, Lactic Acid Bacteria (LAB) Products, and Their Mixture. (1) untreated *K. pneumoniae*. (2) *K. pneumoniae* treated with resveratrol.(3)K. *pneumoniae* treated with lactic acid bacteria (LAB) products. (4) *K. pneumoniae* treated with a mixture of resveratrol and LAB products

3.5.4. Effect on Swarming Motility

The results of this study showed that all the natural treatments used had varying degrees of inhibitory effects on swarming in *P. mirabilis* compared to the control. Resveratrol exhibited the strongest inhibitory effect on swarming, significantly reducing the swarming diameter of the bacteria on the agar medium, outperforming both lactic acid bacteria products and the combined treatment. In contrast, the natural extracts of lactic acid bacteria showed a moderately strong inhibitory effect, while the combined effect was less pronounced than that of resveratrol alone, suggesting that resveratrol has a higher efficacy in disrupting the mechanisms associated with swarming motility in *P. mirabilis* as shown in Fig.5.

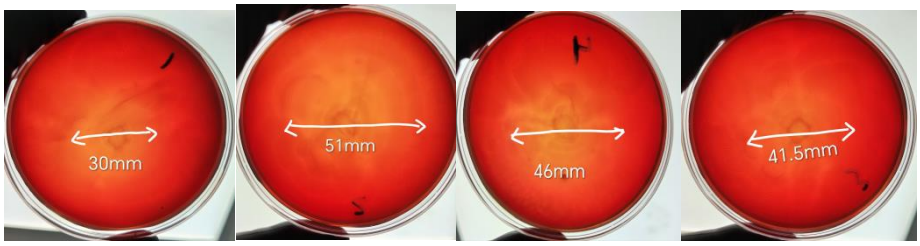

Figure5: The Figure Illustrates the Individual and Combined Effects of Resveratrol and Lactic Acid Bacteria Products on The Swarming of *P. mirabilis*. (1) Culture medium inoculated with bacteria only, (2) Culture medium supplemented with resveratrol and inoculated with bacteria, (3) Culture medium containing lactic acid bacteria products and inoculated with bacteria, (4) Culture medium containing a combination of resveratrol, lactic acid bacteria products, and inoculated with

3.5.5. Effect on Lactose Fermentation

The results showed that untreated E. coli isolates retained their natural ability to ferment lactose, as evidenced by the clear change in the culture medium color to pink, indicative of fermentation. In contrast, treatment of the bacterial isolates with resveratrol alone resulted in a significant change in the culture medium color from pink to light yellow compared to the control group, indicating a clear decrease in the bacteria's ability to ferment lactose. The results also showed that the effect of resveratrol was more pronounced compared to the other treatments used, as the color change in the culture medium was weak or absent.

3.5.6. Effect on Oxidase Production

The results of this study showed that the three tested natural treatments resveratrol, lactic acid bacteria products, and a combination of both did not significantly affect oxidase production in *P. aeruginosa* compared to the control. A positive oxidase test was observed in all bacterial isolates exposed to the treatments, indicating that enzyme activity was unaffected under the experimental conditions. These results suggest that oxidase production remains stable when bacteria are exposed to the natural treatments at sub-inhibitory concentrations.

3.5.7. Effect on Biofilm Production

The results showed that all treatments used, including resveratrol, lactic acid bacteria products, and the combined treatment, led to a significant decrease in biofilm production in isolates produced from the four Gram-negative bacteria causing urinary tract infections, compared to the control group.Both tube and plate methods showed similar results, with a decrease in biofilm density observed after treatment, represented by reduced staining or weaker adhesion to the inner walls of the tubes and plate surfaces, compared to untreated isolates. Resveratrol alone was found to have a more pronounced effect on biofilm production than the other treatments. The results also indicated a variation in the degree of response among the studied bacterial species, with *E. coli* isolates exhibiting the greatest decrease in biofilm production compared to the other species Fig.6.

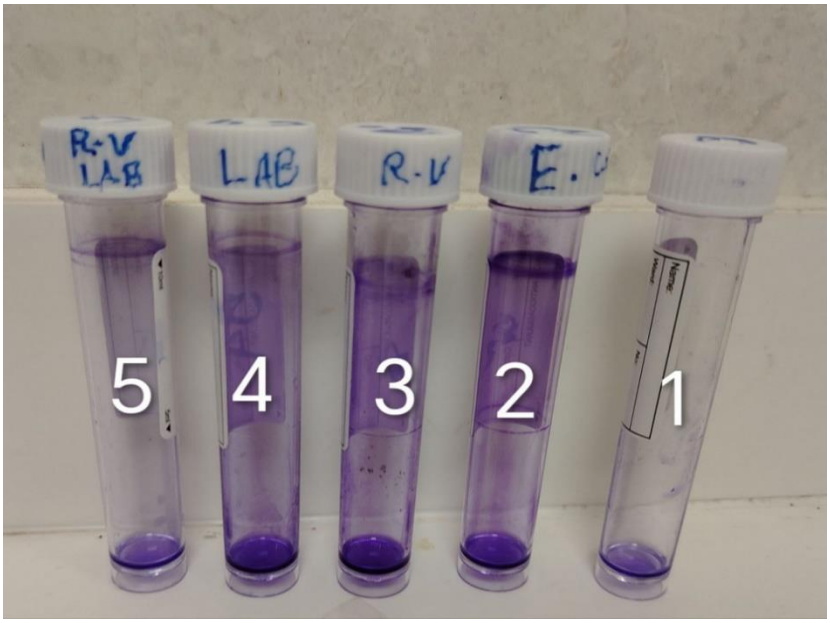

Figure6: Effect of Treatments on *E. Coli* Biofilm Formation: (1) medium only. (2) untreated *E. coli.* (3) *E. coli* + resveratrol. (4) *E. coli* + LAB products. (5) *E. coli* + resveratrol + LAB products**.**

3.6. Effect on Phenotypic Modulation of Antibiotic Resistance

The results showed that treatment with resveratrol, lactic acid bacteria products, and a combination of both increased the susceptibility of Gram-negative urinary tract infection isolates to certain antibiotics to which the isolates in the control group were resistant *E. coli* exhibited resistance to ceftriaxone in the control group, while after treatment with resveratrol or the combination treatment, these isolates became partially to fully susceptible to this antibiotic. The results also showed that combined treatment with resveratrol and lactic acid bacteria products was the most effective in restoring antibiotic sensitivity, compared to the individual treatments. A clear decrease in resistance of the isolates to the antibiotic cefazidime was observed. Furthermore, the inhibition diameters of nitrofurantoin, amikacin, and tigocycline increased with the different treatments compared to the antibiotic alone. These results indicate that the three treatments can alter the susceptibility pattern to antibiotics in previously resistant bacteria, thus enhancing their potential use as adjuvant agents to reduce resistance Fig.7.

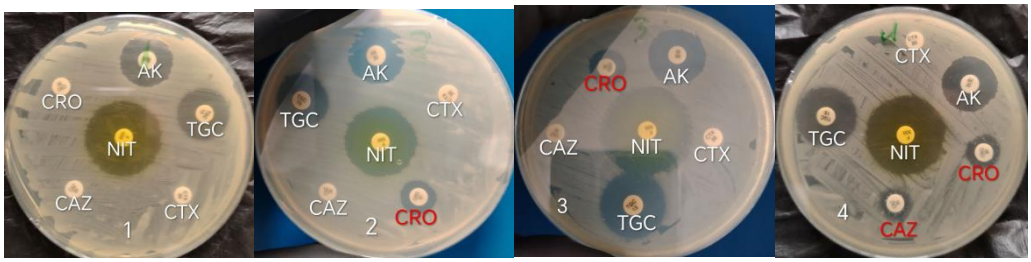

Figure7: In Vitro Evaluation of The Antibacterial Response of Treated and Untreated Bacterial Isolates to Selected Antibiotics. (1) Untreated Isolates**.** (2) resveratrol-treated isolates. (3) LAB products–treated isolates. (4) isolates treated with a mixture of resveratrol and LAB products.

Table 2: Clinical Breakpoints for Antibiotics Used Against Bacterial Isolates According to CLSI 2025

Antibiotic	Concentration (µg/disc)	Sensitive S (mm)	Intermediate I (mm)	Resistant R (mm)
Cefaxim	30	≥19	16-18	<16
Cefotaxime	30	≥26	23-25	<23
Ceftriaxone	30	≥23	20-22	<20
Levofloxacin	5	≥22	18-21	<18
Ciprofloxacin	5	≥22	18-21	<18
Trimethoprim	25	≥16	11-15	<11
Clotrimazole	25	≥16	11-15	<11
Gentamicin	10	≥15	-	<15
Cefepime	30	≥18	15-17	<15
Nitrofurantoin	300	≥17	15-16	<15
Ceftazidime	30	≥18	15-17	<15
Amikacin	30	≥19	16-18	<16
Meropenim	10	≥19	16-18	<16
Colistin	10	N/A		
Tigecycline	15	≥19	15-18	<15

4. Discussion

This study observed a prevalence of multidrug-resistant isolates (MDR) of 86.95%. The indiscriminate use of multiple courses of antibiotic treatment for asymptomatic or mildly symptomatic urinary tract infections (UTIs) is likely the cause of the development of MDR isolates (AL-Khikani, Abdullah and Karkaz, 2024). The results of this study reveal a relatively higher prevalence of resistance in urinary tract pathogenic E. coli (UTIs) compared to other previous local studies. A previous study by Abed and Moter(Abed and Mutter, 2023) reported a resistance prevalence of 87.8% in UPEC E. coli, which is comparable to our current study, confirming that Gram-negative bacteria are highly resistant and pose a real threat to human health. Women are the most susceptible group to harboring MDR bacteria during UTIs, including fluoroquinolone-resistant UTIs and broad-spectrum κ-lactamase-resistant Enterobacteriaceae (Al-kabi and Owaif, 2025).

The results showed that resveratrol possesses antibacterial activity that can affect bacterial growth and reproduction rates. Evidence suggests that this antimicrobial effect is not only related to the general inhibition of bacterial cell growth but also involves molecular mechanisms that influence essential biological processes in bacteria. One of the most prominent mechanisms observed in studies is that resveratrol can inhibit bacterial cell division by suppressing the expression of proteins crucial to cell division, such as FtsZ, a protein essential for the formation of the Z-ring, which allows for proper bacterial division. When this protein is inhibited, cells effectively cease dividing, leading to delayed or inhibited bacterial growth and a consequent reduction in bacterial population growth in the culture medium.(Bejenaru *et al.*, 2024).

Lactic acid bacteria (LABs) play an important role in inhibiting the growth of pathogenic bacteria by producing a range of antimicrobial compounds. These compounds include organic acids such as lactic and acetic acid, which lower the pH of the medium. This leads to the release of protons within bacterial cells and inhibits the activity of acid-sensitive enzymes, thereby inhibiting growth or killing bacteria sensitive to these acidic conditions. Some LAB strains also produce antimicrobial proteins known as bacteriocins, which are biologically active molecules that attack the cell membranes of targeted bacteria or disrupt cell wall synthesis. They create pores in the cell membrane, causing the cell to lose its ion and energy balance, which in turn inhibits growth or leads to cell rupture in many pathogenic bacteria. Therefore, LAB organic acid and bacteriocin products are effective agents in controlling the growth of unwanted microbes (Aman *et al.*, 2021).

Resveratrol is a naturally occurring polyphenolic compound with antibacterial effects. It directly and indirectly influences gene expression associated with hemolysin production, capsule formation, and swarming motility(Inchingolo *et al.*, 2022). Studies have shown that resveratrol inhibits hemolysin secretion by disrupting clotting pathways, thereby reducing the expression of genes responsible for hemolytic toxin synthesis. This, in turn, reduces the bacteria's ability to cause tissue damage and evade the host's immune response. Resveratrol also contributes to reducing bacterial capsule formation by inhibiting metabolic pathways involved in polysaccharide production. This weakens bacterial resistance to phagocytosis and reduces their ability to adhere and form biofilms (Ai *et al.*, 2022). Regarding the swarm phenomenon, resveratrol inhibits this flagellated collective movement by reducing the gene expression of motor proteins and interfering with clonal sensing signals, thereby limiting bacterial spread on surfaces and reducing their ability to colonize tissues and cause infection (Ghaidaa, Yanchang and Hindi, 2014). Thus, resveratrol shows promise as an inhibitor of bacterial virulence factors without directly affecting bacterial growth, making it a potential option in alternative strategies for combating multidrug-resistant bacteria. The results of this study indicate that lactic acid bacteria products possess a clear ability to inhibit key virulence factors in Gram-negative bacteria causing urinary tract infections. This effect is attributed to these products interfering with the regulatory mechanisms responsible for gene expression of virulence factors, rather than directly affecting bacterial growth(Colautti *et al.*, 2022). The marked decrease in hemolysin production is explained by the ability of organic acids and bacteriocins produced by LAB to disrupt quorum sensing systems, particularly those dependent

on N-acyl homoserine lactones (AHLs), which play a pivotal role in regulating genes responsible for hemolytic toxins(Prazdnova *et al.*, 2022).The reduction in capsid formation may also result from the inhibition of metabolic pathways associated with polysaccharide synthesis, thus reducing the bacteria's ability to resist phagocytosis and evade the immune response within the urinary tract(Aman *et al.*, 2021). The reduction in swarming is attributed to the effect of LAB metabolites on the gene expression of flagellar-associated motor proteins, as well as the disruption of cellular signaling necessary for the collective regulation of motility—a characteristic linked to bacterial dispersal and colonization . (Yahya, Aazmi and Ismail, 2025). Similarly, the inhibition of biofilm formation is attributed to the cumulative effect of reducing swarming and capsid formation and interfering with QS signaling, which weakens the initial adhesion and stability of bacterial communities on biological surfaces (Koodi, Najeeb and Abdul, 2025). These findings are particularly relevant in the context of urinary tract infections, where biofilms and their associated virulence factors are major drivers of infection persistence and increasing antibiotic resistance, thus supporting the potential role of LAB products as promising adjuvants in strategies to reduce the virulence of Gram-negative bacteria. Resveratrol acts as an antibiotic resistance modulator by inhibiting biofilm formation and interfering with gene expression associated with virulence and resistance factors. This is explained by increased outer membrane permeability and reduced bacterial ability to form protective biofilm communities(Li *et al.*, 2025). Another effect of resveratrol is its ability to disrupt membrane-dependent resistance mechanisms by increasing membrane permeability, potentially restoring bacterial sensitivity to antibiotics (Wang *et al.*, 2023).Furthermore, resveratrol can act as a potent inhibitor of excretory pumps such as NorA in Staphylococcus aureus, leading to increased intracellular antibody accumulation and enhanced therapeutic efficacy(Santos *et al.*, 2023). Therefore, using resveratrol in conjunction with LAB products can be considered a promising strategy for developing natural antibiotic alternatives or enhancers, provided that extensive molecular and clinical studies are conducted to determine optimal dosages and ensure biosafety.

Conclusions

In our study, *E. coli* was the most common pathogen in Gram-negative (UTIs), accounting for 56.5% , followed by *K. pneumoniae* (21.7%), *P. mirabilis* (8.6%), and *P. aeruginosa* (13%). The majority of Gram-negative isolates were resistant to antibiotics. Resveratrol and a cell-free extract of Lactobacillus bacteria demonstrated significant activity against the growth of Gram-negative bacteria. The study also showed that the natural extracts significantly affected some morphological characteristics of the bacterial isolates, reducing hemolysin production, capsule formation, and swarming motility. A significant decrease in biofilm production was also observed in the tested bacterial isolates, reflecting their effect in reducing important virulence factors. It was also observed that bacteria regained their sensitivity to some antibiotics after becoming resistant to them when treated with natural extracts.

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