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Review

# Evaluation of the antibacterial activity of Weissella confusa K3 cell-free supernatant against extended-spectrum βeta lactamase (ESBL) producing uropathogenic Escherichia coli U60

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ABSTRACT

Different strategies have been approved for controlling extended-spectrum βeta lactamase (ESBL) producing uropathogenic bacteria. The antibacterial activity of Lactic acid bacteria (LAB) is an effective strategy due to its probiotic characteristics and beneficial effects on human health. The antibiotic susceptibility test, disk diffusion method, and double disc synergy test indicated that five enteric uropathogenic isolates were ESBL producers during the present study. They recorded diameters of inhibition zones as ≤ 18, ≤ 8, ≤ 19, and ≤ 8 mm against cefotaxime (CTX), ceftazidime (CAZ), aztreonam (ATM), and ceftriaxone (CRO). Genotypically, $bla_{TEM}$ genes are the most common, with (100 %) occurrence in all the five enteric tested uropathogens, followed by $bla_{SHV}$ and $bla_{CTX}$ genes (60 %). In addition, out of 10 LAB isolates from dairy products, the CFS of isolate no. K3 had high antibacterial activity against the tested ESBLs, especially no. U60, with a MIC of 600 µl. Additionally, the MIC and sub-MIC of K3 CFS inhibited the production of antibiotic-resistant $bla_{\ TEM}$ genes of U60. Analyzing the 16S rRNA sequence confirmed that the most potent ESBL-producing bacteria (U60) and LAB (K3) isolates were identified as *Escherichia coli* U60.1 and *Weissella confuse* K3 with accession numbers MW173246 and MW173299.1, respectively, in GenBank.



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*Abbreviations:* **LAB**, *lactic acid bacteria*; **ESBL**, extended-spectrum beta-lactamase; **MRS**, De Man, Rogosa, and Sharpe; **UTI**, Urinary tract infection; **MRD**, Multidrug resistance; **MHA**, Mueller–Hinton agar; **WC K3**, *Weissella confusa* K3; **ECU60**, *Escherichia coli* U60; **U**, Urine sample.

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## 1. Introduction

Antibiotic use is thought to contribute to the emergence of antimicrobial resistance. Antibiotic overuse has been demonstrated to affect the prevalence of resistant bacteria. As a result, antimicrobial stewardship initiatives and antibiotic management should be implemented to limit antimicrobial resistance (Kim et al., 2021).

Urinary tract infection (UTI) is one of the most common illnesses in the community and one of the leading causes of gram-negative bacteria in hospitalized patients (Lalueza et al., 2018). The primary agents of UTIs, the most commonly encountered microorganisms, are *Enterobacteriaceae*, including *E. coli* (Flores-Mireles et al., 2015), *Klebsiella. pneumoniae*, and *Enterobacter* spp. (Gupta et al., 2001).

Extended-spectrum β-lactamases (ESBL) infections increase; therefore, the resistance of E.S.B.L-producing increases (Khety et al., 2017). Because of the development of resistance to most antibiotics and the difficulties of treatment, the increase in (ESBL)-producing bacteria has become a global issue (Gharavi et al., 2021).

Antibiotic exposure promotes the colonization of the gut by ESBL-producing *Enterobacteriaceae* (ESBL-PE) (Chenouard et al., 2021). ESBL enzymes readily hydrolyze penicillins and cephalosporin, and yet clavulanic acid can inhibit them (Rawat and Nair, 2010). There are various variations that encode these hydrolytic enzymes. TEM (Temoniera), CTX-M (Cefotaximase-Munich), SHV (Sulfhydryl variable), and OXA (Oxacillin) are essential for identifying ESBL molecularly. They are typically mobile, found on plasmids, and allow horizontal transmission (Ur Rahman et al., 2018). These mobile elements in these plasmids carry resistance to additional drug classes, including sulphonamides, aminoglycosides, and fluoroquinolones. As a result, the bacteria that contain these plasmids are frequently multidrug-resistant (Zeynudin et al., 2018). Diseases caused by ESBL-producing bacteria have restricted treatment options, contributing to a high fatality rate (Hawkey et al., 2018).

*Lactobacillus*, *Bifidobacterium*, and *Enterococcus* spp. are LAB bacteria found in probiotic supplements. *Pediococcus*, *Streptococcus*, *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Lactobacillus*, and *Weissella* are among the most common food-related LAB (Zheng et al., 2020), included in the phylum *Firmicutes* (Fesseha et al., 2021).

LAB is mainly found in nutrient-rich environments. They are found in human and animal vaginal and gastrointestinal tracts (GIT). Moreover, they can be found in fish, vegetables, meat, and dairy products (such as cheeses, kefir, and milk and meat as part of the nonstarter microbial populations) (Mokoena, 2017; Ramos et al., 2020).

LAB has the potential as a probiotic, which could help manage pathological disorders by altering the intestinal microbiota of patients taking antibiotics or as therapy adjuvants during oral administration (Varzakas, 2020). They contribute to minimizing the use of antibiotics (Mokoena, 2017), or can be employed as alternatives (Ramos et al., 2020).

Furthermore, LAB can produce compounds such as bacteriocins, lactic acid, vitamins, fatty acids, amines, and exopolysaccharides used in the probiotic functions and fermentation industry. LABs can be used as probiotics to promote health for pathogen control (Wang et al., 2021), health-promoting applications in the agroindustry, and preservation (Mokoena, 2017). In addition, LAB peptides, such as bacteriocins, are utilized against harmful microbes that cause various disorders (Rakhmanova et al., 2018).

The antibacterial activity of LAB isolates against *K. oxytoca*, *E. coli*, *Salmonella*, and *Staphylococcus intermedius* was demonstrated in-vitro (Dowarah et al., 2018).

*Weissella* spp. appears to have a wide range of technological and functional capabilities to improve product safety and sensory characteristics (Fessard and Remize, 2017). A gram-positive facultative anaerobe with fermentative metabolism makes up the genus *Weissella*. The strains of this genus have been isolated from various ecological settings, including fermented food (López-Hernández et al., 2018).

We need to define the mechanisms of action and determine the ideal dosage for multistrain probiotics. Probiotics may replace growth regulators, which banned by the European Union in 2006. Moreover, they improve health, and overcome the resistance problems and a concern with the world's public health (Jha et al., 2020; Kazemi et al., 2019).

### 1.1. Contributions: The following are our contributions to this study

i) We tested WC K3′s antibacterial and anti-ESBL properties against the uropathogen ECU60. Our study demonstrated that WC K3 isolated from old fermented kareesh cheese was the most effective natural bio-control agent with notable antimicrobial and ESBL enzyme activities against ECU60.

ii) WC K3 prevented the expression of the ESBL bla $_{TEM}$ gene by ECU60. Because of this, WC K3 could have a wide range of applications in the medical industry as an antibacterial agent and as a protective agent to regulate and stop uropathogens. In-vitro testing was carried out in this study to see if it might be used in real-world situations.

## 2. Materials and methods

### 2.1. Food specimen

Twenty commercial dairy product samples were collected from different sources (eight samples from pickles, six samples from

**Table 1**
Critical oligo primers.

| | Primer sequences (5'-3') | Length of amplified product | Reference |
|---|---|---|---|
| $Bla_{TEM}$ | ATCAGCAATAAACCAGC<br>CCCCGAAGAACGTTTTC | 516 bp | (Colom et al., 2003) |
| Bla $_{SHV}$ | AGGATTGACTG.C.CTTTTTG<br>ATTTGCTGATTTCGCTCG | 392 bp | |
| Bla $_{C.T.X}$ | ATG TGC AGY ACC AGT AAR GTK ATG GC<br>TGG GTR AAR TAR GTS ACC AGA AYC AGC GG | 593 bp | (Archambault et al., 2006) |

yogurt, and six from old kareesh cheese) for the LAB isolation. These samples were obtained between March 2019 and December 2019 from various markets in the Sharkia Governorate of Egypt.

### 2.2. Isolation and characterization of lactic acid bacteria

About 25 g of each food sample was serially diluted with a saline solution of NaCl 0.85 % at 25 °C. Each serial dilution (0.1 ml) from each food specimen has then coated the surface of De Man, Rogosa, and Sharpe 1960 (MRS) agar plates according to the method described by Shukla et al. (2008). The same media were used for purification after a 24-hour incubation period at 35 °C with the plates. Growth, cell morphology, gram staining, and catalase activity of the produced LAB isolates were assessed during purification and testing. Additionally, identification was made using LAB characteristics listed in Bergey's Manual of Determinative Bacteriology, patterns of carbohydrate fermentation, incubation times at various temperatures in MRS agar, and other factors (Garrity et al., 2004). Based on earlier assays, isolates were then chosen for additional identification, such as growth at various pH values, tolerance to NaCl, and milk agglutination (Harrigan and McCance, 1976).

### 2.3. Isolation of pathogenic enteric bacteria

Uropathogens (50 isolates) were obtained from urine samples of patients suffering from UTI at Zagazig University Hospitals from November 2017 to September 2018. McConkey agar medium was used for enteric bacterial isolation (Murray et al., 2007).

Based on their physical and biochemical properties, these collected uropathogens were identified (Holt et al., 1994). According to the Clinical and Laboratory Standards Institutes' (CLSI, 2012) recommendations, 13 antibiotic discs were used in the antibiotic sensitivity test for all isolates.

### 2.4. Determination of ESBL production

2.4.1 Phenotypic detection of ESBL was carried out using the antibiotics aztreonam, ceftriaxone, ceftazidime, cefotaxime, and amoxicillin-clavulanic acid by the disk diffusion method and double-disc synergy test (CLSI, 2021).

#### 2.4.2. Genotypic detection of E.S.B. Ls ($bla_{TEM}$, $bla_{S.H.V}$, $bla_{C.T.X}$ Genes)

The DNA was extracted according to QIAamp DNA. Mini kit instructions. Using oligonucleotide primer sequences, pathogenic isolates were subjected to ESBL detection by PCR, as shown in (Table 1).

This led to the selection of *E. coli* (U27&60), *K. pneumonia* (U52&65), and *P. eruginosa* (U10) for antibacterial activity assays that are the most MDR ESBLs producer of uropathogens.

### 2.5. Antibacterial assay of LAB against ESBL

The antibacterial activity of CFS of LAB isolates against *E. coli* (U27& 60), *K. pneumonia* (U52& 65), and *P. aeruginosa* (U10) was evaluated using the agar well diffusion technique. Each overnight LAB culture (aged 24 h) was inoculated into 100 ml of MRS broth. The mixture was incubated for 48 h at 37 °C in a shaking incubator to produce a cell-free supernatant. After incubation, the cultures were centrifuged at 5000 g for 10 min at 4 °C. After removing the cells from each LAB isolate, the CFS was then extracted. Each isolate's CFS was brought to pH 6.5 using 1 M NaOH to prevent the inhibitory effects of acid before being sterilized with a 0.22 mm Millipore filter. The Muller-Hinton agar plates surface were inoculated with the targeted MDR *E. coli* (U27&60), *K. pneumonia* (U52&65), and *P. aeruginosa* (U10) (106 CFU/mL) (Schillinger and Lücke, 1989). Sterile cork poorer was used to create 6 mm-diameter wells, and 50 L of CFS was added to each well. The plates were maintained at 37 °C for 24 h, and the diameter of the inhibitory zone (mm) was measured (Reda, 2019).

### 2.6. Molecular identification of selected bacteria

Molecular identification was performed for the selected ESBL uropathogen ECU60 and the most potent LAB isolate (Freeman et al., 1990; Kolbert and Persing, 1999; Zhang et al., 2004). An examination of 16S rRNA gene sequences validated the identification. The PCR-mediated amplification of the 16S rRNA and purification was performed using PrepMan Ultra (Applied Biosystem), Microseq PCR, and Microseq Cycle Sequencing (Applied Biosystems). The polymerase chain reaction (PCR) was used to amplify the 16S rRNA using the universal primers: 1492R (5′-CGGTTACCT TGTTACGACTT-3′), and 8F (5′-AGAGTTTGAT CCTGGCTCAG-3′). The sequences of 16S rRNA were uploaded to the NCBI website

**Table 2**
Phenotypic confirmation test for ESBL.

| Bacterial isolates no. | ATM | | CTX | | CAZ | | CRO | | AMC |
|---|---|---|---|---|---|---|---|---|---|
| U10 | 5 | R | 5 | R | 5 | R | 5 | R | 18 |
| **U27** | 17 | I | 15 | I | 5 | R | 15 | I | 16 |
| **U52** | 19 | I | 18 | I | 8 | R | 8 | R | 17 |
| **U60** | 5 | R | 5 | R | 5 | R | 5 | R | 14 |
| **U65** | 5 | R | 5 | R | 5 | R | 5 | R | 15 |

R= Resistant, I= Intermediate, and S= Sensitive.

**Table 3**
The detection of ESBL by PCR in ESBL-producing bacteria.

| Bacterial isolate no. | E.S.B.L | | |
|---|---|---|---|
| | ***bla*$_{TEM}$** | ***bla*$_{S.H.V}$** | ***bla*$_{C.T.X}$** |
| U 10 | + | + | + |
| U 27 | + | - | - |
| U 52 | + | - | - |
| U 60 | + | + | + |
| U 65 | + | + | + |
| % of occurrence | 100 | 60 | 60 |

(https://www.N.C.B.I.nlm.nih.gov). The basic local alignment search tool was used to analyze sequences and compare them to published sequences (BLAST) (https://blast.N.C.B.I.nlm.nih.gov/Blast.cgi), and the submission was conducted in GenBank.

### *2.7. Assay of anti-bacterial effect of L.A.B K3 against selected E.S.B.L producing bacteria*

MICs of LAB against selected ESBL-producing bacteria. During this experiment, a modified turbidity method was used (Schwalbe et al., 2007). Different concentrations of LAB K3 CFS (0 – 1000 µl) were added separately to 24 h aged (106 CFU/mL) tested ESBL isolates (U 10, 27, 52, 60 &65) in test tubes to a total volume of 2 ml with nutrient broth. After 24 h of incubation at 37 °C, the MIC was determined to be the lowest dose of an antibacterial agent that prevented the organism in the tubes from growing visibly (Goldstein et al., 1978).

As a result, E. coli U60 was chosen as the ESBL uropathogen-producing isolate to serve as the indicator organism. The MIC and sub-M.I.C of K3 CFS against *E. coli* U60 were used to detect bla$_{TEM}$ genes by PCR.

## 3. Results

### *3.1. Phenotypic detection of ESBLs producing bacteria*

Five isolated enteric bacteria were characterized according to their gram stain and biochemical tests as *E. coli* (U27 & 60), *K. pneumonia* (U52 & 65), and *P. aeruginosa* (U10). The results of the antibiotic sensitivity test against 13 disks indicated that the tested isolates were susceptible to amikacin (AK), and 100 % of isolates were resistant to clindamycin (DA) and (CAZ) (data not shown).

The resistance profile of ESBLs was carried out using the disk diffusion method and double disc synergy test. The phenotypic detection of five enteric bacteria is explained in Table 2. The results indicated that all isolates had inhibition zones of diameters $\leq$ 18 mm for CTX, $\leq$ 8 mm for CAZ, $\leq$ 19 mm for ATM, and $\leq$ 8 mm for CRO. All isolates were sensitive to AMC.

### *3.2. Genotypic detection of E.S.B. Ls (bla$_{TEM}$, bla$_{S.H.V}$, and bla$_{C.T.X}$ Genes)*

The isolates no. (U 10, 27, 52, 60, and 65) were suspected to be ESBL producers and subjected to PCR for genotypic confirmation by

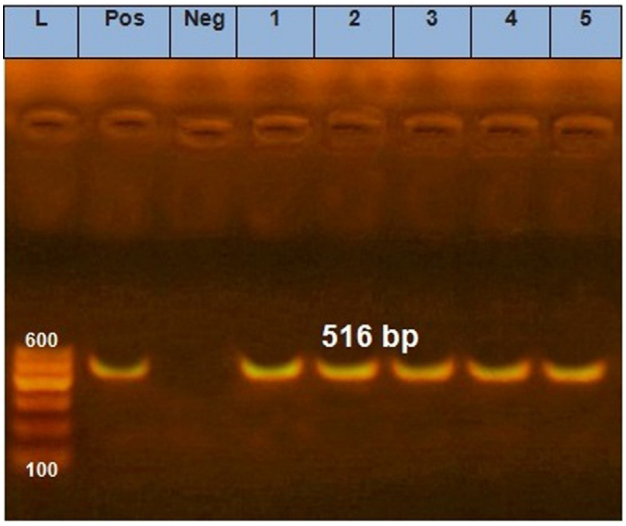


**Fig. 1.** PCR detection of ESBL gene $bla_{TEM}$. Isolates no. 10, 27, 52, 60, and 65 were positive. Lanes 1, 2,3,4, and 5 represent isolate no. 10, 27, 52, 60, and 65, respectively. Lanes Pos. and Neg. for positive and negative controls. Lane L for DNA ladder Marker 100–600 bp.

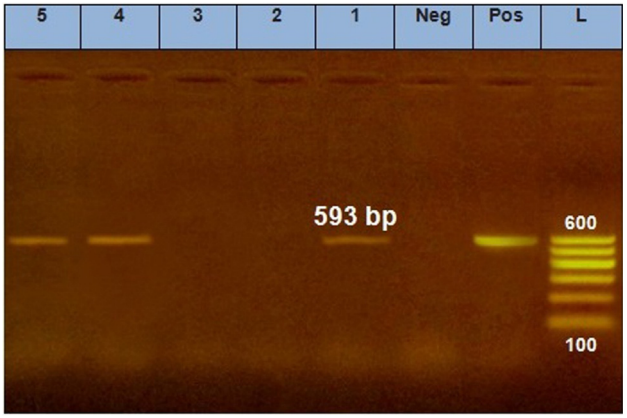


**Fig. 2.** PCR detection of ESBL gene $bla_{C.T.X}$. Lanes 1, 2, 3, 4, and 5 correspond to isolates 10, 27, 52, 60, and 65, respectively. Lanes Pos. and Neg. for positive and negative controls. Lane L for DNA ladder Marker 100–600 bp.

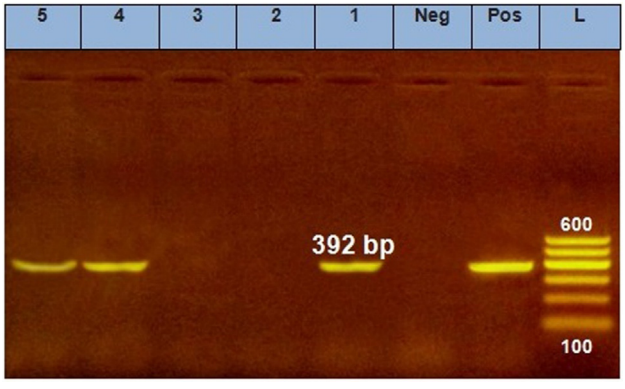


**Fig. 3.** PCR detection of ESBL gene $bla_{SHV}$ Lanes 1, 2, 3, 4, and 5 for isolates no. 10, 27, 52, 60, and 65, respectively. Lane Pos. and Neg. for positive and negative controls. Lane L for DNA ladder Marker 100–600 bp.

searching for the genes $bla_{TEM}$, $bla_{S.H.V,}$ and $bla_{C.T.X}$. All five isolates had $bla_{TEM}$. However, isolates no. (U 10, 60, and 65) also had $bla_{S.H.V}$, $bla_{C.T.X,}$ as shown in Table 3 and Figs. 1, 2, and 3. Additionally,

**Table 4**
Antibacterial activity of CFS of LAB K3 against ESBL.

| E.S.B.L | Diameter of inhibition zone (DIZ), mm |
|---|---|
| *Pseudomonas. sp (U10)* | **11 ± 0.5** |
| *E. coli (U27)* | **12 ± 0.5** |
| *Klebsiella. sp (U52* | **14 ± 0.5** |
| *E. coli (U 60)* | **15 ± 0.5** |
| *Klebsiella. sp (U65)* | **13 ± 0.5** |

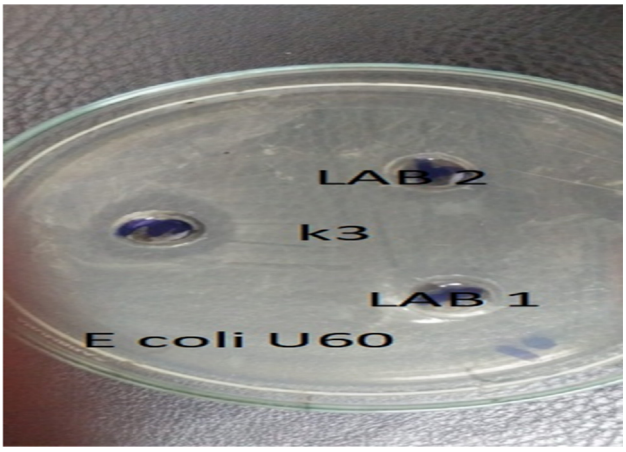


**Fig. 4.** Antibacterial activity of selected LA B against ESB L producing *E. coli* U60.

the results indicated that $bla_{TEM}$ genes were the most prevalent, with 100 % occurrence in all tested ESBL-producing bacteria, followed by $bla_{S.H.V}$ and $bla_{C.T.X}$ (60 %).

### *3.3. Antibacterial activity of selected L.A.B isolates against E.S.B.L producing bacteria*

Ten isolates were positive for their growth on MRS agar plates with their characteristic colonies. The CFS of each LAB isolate was tested for antibacterial activity against isolated ESBL producers U (10, 27, 52, 60, and 65) using the well diffusion method. It was found that, out of all tested LAB, isolate K3 exhibited antibacterial activity against ESBL-producing enteric bacteria isolate no. U (10, 27, 52, 60, and 65). The highest activity was against (U60), which had a 15 mm inhibition zone diameter (Table 4) and (Fig. 4).

### *3.4. Characterization of the selected LAB isolates K3*

The selected K3 isolate was characterized as gram-positive, short rods, and catalase-negative as the genus *Weissella (*data not shown). Tolerances of NaCl, growth at temperatures, milk coagulation, and tests for resistance to low pH were illustrated in (Table 5). The results showed that LAB K3 tolerated a high salt concentration as the main habitat and coagulated milk after 6 h. It survived at different temperatures, with the best growth at 37 °C for 48 h and at 45 °C for 48 h and 72 h. Additionally, K3 survived at pH 3 as the pH of the gut, and the best growth was at pH 6 for 24 h at 37 °C.

### *3.5. Anti-bacterial effect of L.A.B K3 against selected E.S.B.L producing bacteria*

MIC of LAB isolate *(K 3) CFS* against ESBL-producing bacteria was tested. In these assays, the most effective LAB (K3) CFS was used against ESBL-producing bacteria: *P. aeruginosa* (U10), *K. pneumoniae* U (52, 65), and *E. coli* U (27, 60). The MICs values were determined as shown in (Fig. 5). The results demonstrated that 800 µl of LAB CFS is the MICs against *P. aeruginosa*(U10),

**Table 5**
The effect of different NaCl %, milk coagulation, pH %, and temperatures on selected LA B isolates (K3).

| Test | Growth | | |
|---|---|---|---|
| **NaCl concentration %** | | | |
| 9.0 | + | | |
| 8.5 | + | | |
| 8.0 | + | | |
| 7.5 | ++ | | |
| **Milk coagulation (h)** | | | |
| 6 | + | | |
| 12 | + | | |
| 24 | + | | |
| **pH concentration (%)** | | | |
| 3.0 | + | | |
| 4.0 | + | | |
| 6.0 | ++ | | |
| **Temperature °c** | The incubation period (h) | | |
| | 24 | 48 | 72 |
| 15 | + | + | + |
| 37 | + | ++ | ++ |
| 45 | + | ++ | ++ |

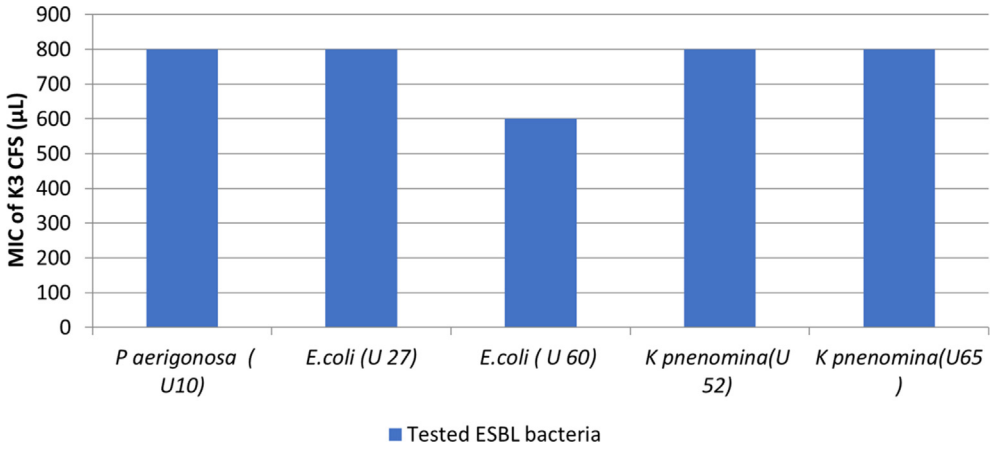


**Fig. 5.** The MIC of LAB isolates (K3) CFS against ESBL-producing bacteria ($bla_{TEM}$) of *E. coli* U (60) treated with LAB (K3).

*K. pneumoniae* (U52 & U65), and *E. coli* (U27), and it is 600 µl for *E. coli U* (60).

This experiment was conducted to ensure the effect of CFS of K3 on the $bla_{TEM}$ of *E. coli* U60. As shown in Fig. 6, the result illustrated that the nontreated ESBL could produce ESBL $bla_{TEM}$, while it could not be produced in the presence of MIC sub-M.I.C of LABK3 CFS.

### 3.6. Molecular characterization

The 16S rRNA gene was amplified by PCR to identify the LAB K3 and ESBL U60 isolates as *Weissella confusa* K3 and *Escherichia coli* U60, respectively. On the NCBI website (https://www.N.C.B.I.nlm.nih.gov), the amplified genes from *W. confusa* K3 and *E. coli* U60

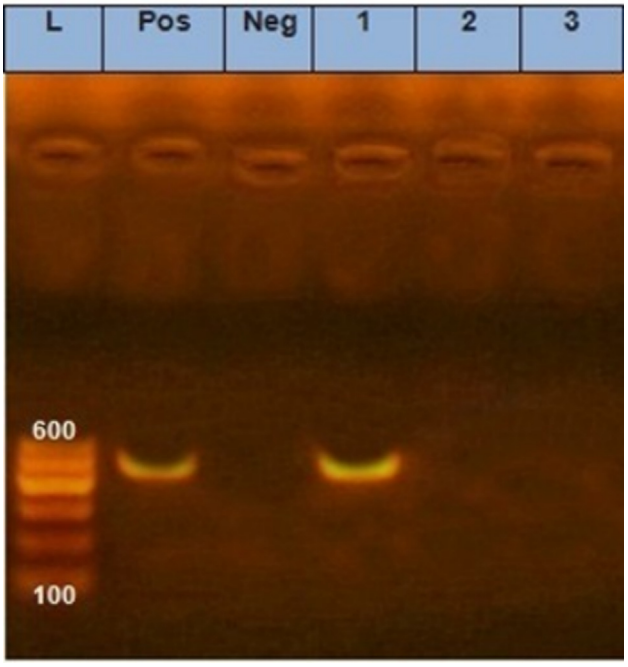


**Fig. 6.** PCR of ($bla_{TEM}$) in ESBL-producing bacteria treated with CFS of K3.Lanes 1, 2, and 3 are for nontreated U60, MIC, and Sub MIC, respectively. Lanes Pos. and Neg. are for positive and negative controls, respectively. Lane L is for DNA ladder Marker 100–600 bp.

had their partial nucleotide sequences submitted to GenBank along with accession numbers (MW173299.1, and MW173246.1). The phylogenetic trees are shown in (Figs. 7 and 8).

## 4. Discussion

Lactic Acid Bacteria (LAB) are well-known probiotics that have been shown to improve human health. The benefits of LAB for both human and animal health and industry have been mentioned in several reviews (Mokoena, 2017; Ramos et al., 2020). One of the essential probiotic properties is their antibacterial action. The most common bacterial illnesses in community settings are urinary tract infections (UTIs) (Lalueza et al., 2018). ESBL infections are more distributed (Khety et al., 2017). In vitro, LAB can inhibit MDR bacteria (Mokoena, 2017).

The current research revealed that the CFS of *Weissella K*3 inhibits the growth of ESBL-producing *E. coli* and prevents its formation of $bla_{TEM}$ genes. LAB isolate no. K3 demonstrated high antibacterial activity against all tested ESBL-producing uropathogens using the well diffusion method. Isolate no. K3 was characterized as a species of the genus *Weissella. Spp* (Garrity et al., 2004). It was found that 800 ml and 600 ml of *W. spp.* CFS were the MIC against (U10, 27, 52, and 65) and *E. coli* U60, respectively. This explained the sensitivity of U60 to LAB K3 more than the other ESBL producers.

Moreover, it was found that K3 affects the formation of $bla_{TEM}$ by E.coli U60 after being treated by CSF of LAB K3. Nevertheless, the antibacterial activity of LAB pathogens might be related to substances such as bacteriocins (Rakhmanova et al., 2018), fatty acids, amines, vitamins, and polysaccharides (Wang et al., 2021), which inhibit microbial growth. In addition, competition for attachment sites, nutrients, alteration of pathogen enzymatic activity, and immunostimulatory functions were other strategies of LAB to inhibit pathogens (Demain and Sanchez, 2009). Bacteriocins might bind to similar receptors on the sensitive surface and kill it. Sensitive mechanisms include the pore-forming type, nuclease types with DNase, RNase, and peptidoglycanase functions (Yang et al., 2014). Similarly, it was reported that antimicrobial agents of

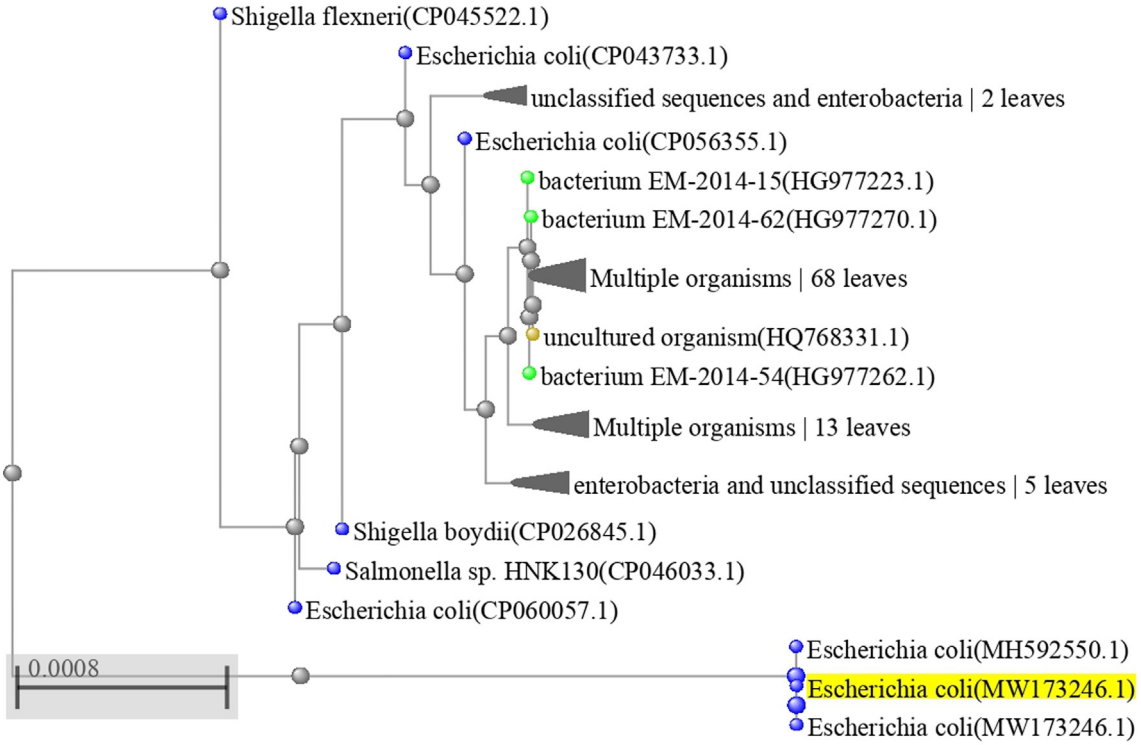


**Fig. 7.** Phylogenetic analysis of *E. coli* U60 (accession no. MW173246.1).

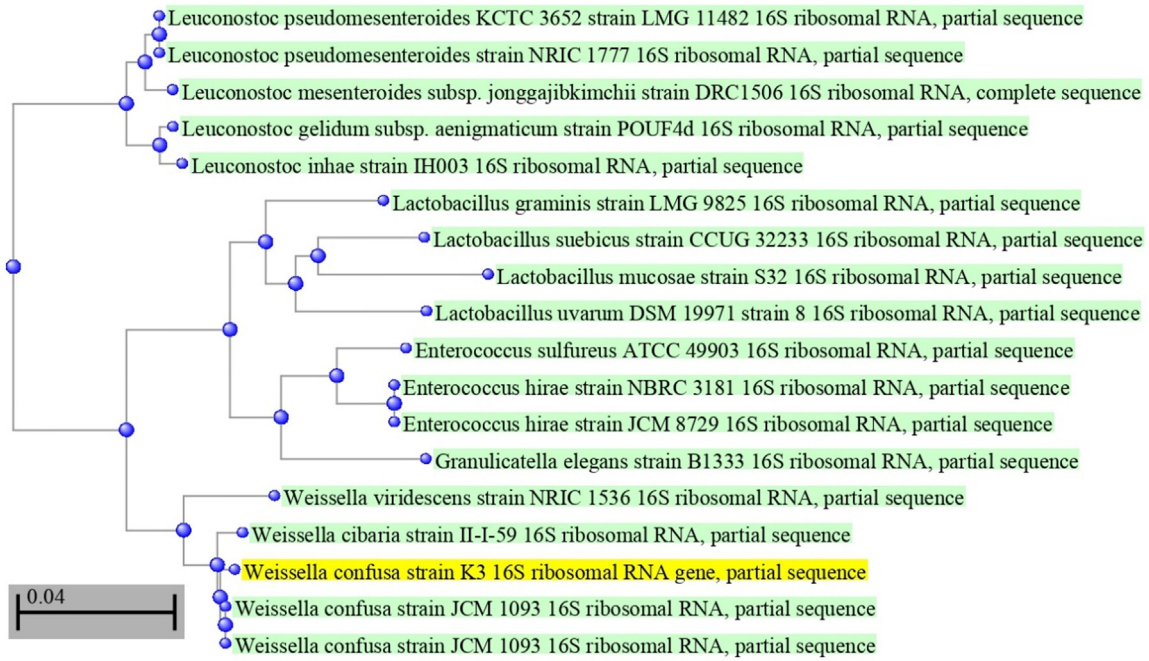


**Fig. 8.** Phylogenetic analysis of *W. confusa* K3 (accession no. MW173299.1).

*W. confusa* strain GCC_19R1 have a considerable zone of inhibition against *Bacillus cereus* strain SN_SA, *A. johnsonii* strain SB_SK, *P. aeruginosa* strain GCC_19W1, *S.maltophilia* strain G.C.C_19 W2, *Cedecea davisae* strain G.C.C_19S1, and *A.spanius* strain G.C.CSB1 (Nath et al., 2020). Additionally, (El-Mokhtar et al., 2020) reported that the CFS of *lactobacilli* demonstrated a high anti-ESBL with inhibition zone diameters greater than 13 mm in the agar well assays against both *K. pneumoniae* and *P. aeruginosa*. Similarly, it was indicated that the CFS of *L. plantarum* Y3 showed a great inhibitory effect against MRD uropathogenic *E. coli* U12 (Mekky et al., 2022).

Five enteric bacteria were isolated from the UTI-infected cases during the present study. They were characterized according to their gram stain and biochemical tests: *E. coli* (U 27&60), *K. pneumonia* (U 52 and 65), and *P. aeruginosa* (U10) (Holt et al., 1994). It was demonstrated that *E. coli* is the most causing UTIs, followed by *K. pneumoniae* (Gharavi et al., 2021; Pandit et al., 2020).

Phenotypically, the five tested uropathogens were characterized as ESBL-producing bacteria using the disk method and double-disc synergy test (CLSI, 2021). The results indicated that phenotypically ESBL is indicated by diameters $\leq$ 18 mm for CTX, $\leq$ 8 mm for CAZ, $\leq$ 19 mm for ATM, and $\leq$ 8 mm for CRO. All isolates were sensitive to AMC. Additionally, it was found that *E. coli* (U 27 and 60) is the most ESBL producer, followed by K. sp (U 52, 65). Consistently, Pandit et al. (2020) reported that *E. coli* is essential, and the majorities (64.9 %) of them are MDR, and among them (40.3 %) are ESBL-producing bacteria. Furthermore, it was reported that among cases with UTI, 30 % are ESBL (Liu et al., 2022). CLS I (2012) noted that ESBL was examined in three categories, ESBL screening method, phenotypic confirmation using a double-disc synergy test, and genotypic detection. The ESBL was indicated when the diameter of inhibition was $\leq$ 27 mm for CTX, $\leq$ 22 mm for CAZ, $\leq$ 25 mm for CRO, and $\leq$ 27 mm for aztreonam (CLSI, 2021). The isolate shows an increase of $\geq$ 5 mm in the zone of inhibition of the combination discs with ampicillin/clavulanic acid was considered an ESBL producer (CLSI, 2015).

Additionally, ESBL-producing isolates no. U (10, 27, 52, 60, and 65) were then subjected to PCR for genotypic confirmation of $bla_{TEM}$, $bla_{S.H.V}$, *and* $bla_{C.T.X}$. All five isolates had been confirmed as ESBL, and all had $bla_{TEM}$, but isolate no. U (10, 60, and 65) had $bla_{S.H.V}$ and $bla_{C.T.X}$ genes. More $bla_{TEM}$ genes were the most prevalent among the isolates with 100 % occurrence, followed by *bla* $_{SH.V}$ *and bla* $_{CTX}$ with 60 %. Previous reviews reported that more than half (54.8 %) of the ESBL-producing *E. coli* isolates harbored *bla-*$_{TEM}$ and *bla-*$_{C.T.X\text{-}M}$ (Jena et al., 2017). Pandit et al. (2020) reported that, among the ESBL genotypes, *bla-*$_{TEM}$ genes (83.8 %) are more prevalent, followed by *bla-*$_{C.T.X\text{-}M}$ (66.1 %) and *bla-*$_{S.H.V}$ (4.8 %) for uropathogenic tested *E. coli*.

ESBL is a heterogeneous group of plasmid-mediated bacterial enzymes, which confirmed the ability to hydrolyze and eliminate a wide variety of β–lactam antibiotics (Bradford, 2001; Xiao et al., 2020). LAB K3 and ESBL U60 isolates were identified as *W. confusa K3 and E. coli U60* by PCR amplification of 16S rRNA. The partial sequences amplified from *W. confusa* K3 and *E. coli* U60 were submitted to GenBank at the NCBI website (https://www.N.C.B.I.nlm.nih.gov) with MW173299.1 and MW173246.1, respectively.

## 5. Conclusions

The current research aimed to study the antibacterial activities and effects of LAB against ESBL uropathogenic producers. The findings revealed the rapid spread of antibiotic resistance. The phenotypic method of ESBL detection should be developed due to the low accuracy caused by the high rate of multidrug-resistant antibiotics. Genotypic methods are the best ESBLs. The antibacterial effect of LAB was investigated in vitro against ESBL-producing uropathogenic bacteria. The findings demonstrated that utilizing CFS of LAB, as an alternative to the antibacterial drug, had a considerable effect against uropathogens and the genetic expression of the antibiotic resistance gene $bla_{TEM}$. This study shows promise in the control of antibiotic-resistant microorganisms. The *W. confusa* K3 strain isolated in this study had antibacterial potential against ESBL-producing enteric uropathogens that can be used in future research in the food and therapeutic industries as probiotics.

## Contributors

RME and NEH collected data, and performed the investigation and practical work. RME, WAH, and AAA designed the data and performed the analysis. RME, NEH, and WAH prepared, wrote, revised, and edited the final manuscript. All authors have approved the final version.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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