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BACTERIOCIN PRODUCTION, ACID-BILE TOLERANCE AND ANTAGONISTIC ACTIVITY OF LOCALLY ISOLATED LACTOBACILLUS SPECIES AGAINST MULTIDRUG-RESISTANT UROPATHOGENS AND ENTEROPATHOGENS

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ABSTRACT

Multidrug-resistant (MDR) bacteria are now responsible for a considerable proportion of urinary and gastrointestinal infections and this has directed research toward antimicrobials from biological origin as alternatives to conventional antibiotics. A total of 45 *Lactobacillus* strains isolated from locally available fermented milk products and vaginal swab samples were used. Phenotypic tests and 16S rRNA gene sequencing placed them in five species: *Lactobacillus plantarum*, *L. rhamnosus*, *L. acidophilus*, *L. fermentum*, and *L. casei*. Every isolate was tested for survival at pH 2.0 and 3.0, for tolerance of 0.3% and 0.5% oxgall, and for bacteriocin production. Cell-free supernatant (CFS) from twelve selected isolates inhibited MDR strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Shigella flexneri* in the agar well diffusion assay. One strain was conspicuous. The greatest results in the overall collection were found for *L. plantarum* LP-7, which maintained 87.2% of its viable count at pH 2.0 after 3 h and 82.5% in 0.3% oxgall. The LP-7 bacteriocin was partially purified by ammonium sulphate precipitation at 70% saturation and showed minimum inhibitory concentration (MIC) values ranging from 25 to 200 µg/mL. Its molecular weight was estimated to be about 6.5 kDa by SDS-PAGE. Thus, local *Lactobacillus* isolates, most all LP-7, seem to be feasible candidates for antimicrobial drugs against MDR uropathogens and enteropathogens.

Keywords: *Lactobacillus*, Bacteriocin, Acid-bile tolerance, Multidrug resistance, Uropathogens, Enteropathogens, Probiotic, Cell-free supernatant.

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INTRODUCTION

UTIs (urinary tract infections) and enteric infections are among the most frequent bacterial diseases in the world. Each year hundreds of millions of people develop them and the toll manifests as disease, treatment expenditures and extensive antibiotic use [1]. The resistance only makes it worse. Multidrug-resistant (MDR) uropathogens and enteropathogens have become common, several first-line antibiotics no longer work against them, and the list of usable drugs keeps shrinking [2]. Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and fluoroquinolone-resistant *Salmonella* spp. are now routine findings in clinical laboratories, which is why new antimicrobial strategies

are needed, and needed quickly [3]. Lactic acid bacteria (LAB), and the genus *Lactobacillus* in particular, are well-documented producers of several antimicrobial metabolites: organic acids, hydrogen peroxide, diacetyl, and bacteriocins. The bacteriocins have drawn the most interest [4]. These are peptides or small proteins made on the ribosome. They kill or arrest closely related bacteria and, in some cases, distantly related ones as well. Most of them act by opening pores in the target cell membrane or by blocking cell wall synthesis [5]. Their spectrum is often narrow, or can be tuned, which sets them apart from broad-spectrum antibiotics and spares more of the resident microbiome [6]. Producing a bacteriocin is not enough on its own. A probiotic candidate also has to pass through the

stomach and the duodenum alive, which means coping with the low pH of gastric juice and with bile salts, whose action on the cell resembles that of a detergent [7]. Acid and bile tolerance are therefore standard selection criteria; a strain that loses most of its viability under these stresses cannot be expected to do anything useful *in vivo* [8]. Published work on *Lactobacillus* probiotics is extensive. Even so, few studies have taken strains isolated locally from traditional fermented foods and measured, in the same set of experiments, both their probiotic fitness and their activity against clinically relevant MDR uropathogens and enteropathogens. That is what we set out to do here. We isolated indigenous *Lactobacillus* spp., measured their acid and bile tolerance, screened them for bacteriocin production, and tested their supernatants against a panel of MDR clinical isolates of urinary and enteric origin.

MATERIALS AND METHODS

Sample Collection and Isolation of *Lactobacillus* spp.

We collected 60 samples of two kinds. The first group was traditional fermented dairy: yoghurt, cheese, and fermented milk (laban) made locally and obtained from open markets and rural households. The second group was high vaginal swab specimens taken from apparently healthy women during routine gynecological check-ups at a local hospital. Sampling ran from September 2025 to February 2026. Each sample was taken aseptically, kept on ice, and reached the laboratory within 2 h. For isolation, one gram or one milliliter of each sample was serially diluted in sterile peptone water (0.1%) up to 10^{-7} . Appropriate dilutions were plated on de Man, Rogosa and Sharpe (MRS) agar (HiMedia, India) supplemented with 0.05% L-cysteine hydrochloride. Plates were incubated at 37 °C for 48 h in an anaerobic jar with gas packs (AnaeroGen, Oxoid). Typical *Lactobacillus* colonies (small, white to creamy, convex, smooth-edged) were chosen, purified by repeated streaking and stored in MRS broth with 20% glycerol at -80°C until required [9].

Phenotypic and Molecular Identification

Presumptive *Lactobacillus* isolates were subjected to a routine battery of phenotypic tests, including Gram stain, catalase, oxidase, motility, and carbohydrate fermentation profiling (API 50 CHL strips, bioMérieux, France). For molecular validation, we amplified the 16S ribosomal RNA gene using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). Genomic DNA was isolated from overnight cultures of MRS broth by commercial kit (Promega, USA). The volume of each PCR was 25 µL. The cycling conditions were 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 55 °C for 45 s and 72 °C for 90 s, followed by a final extension at 72 °C for 7 min. The amplicons were sequenced in both directions by MacroGen Inc. (South Korea). The

sequences were compared with NCBI GenBank database using BLAST [10].

Collection and Antibiotic Susceptibility of Target Pathogens

Six MDR clinical isolates were obtained from the microbiology laboratory of a teaching hospital: *Escherichia coli* (from urine), *Klebsiella pneumoniae* (urine), *Proteus mirabilis* (urine), *Pseudomonas aeruginosa* (urine), *Salmonella typhimurium* (stool), and *Shigella flexneri* (stool). Kirby-Bauer disc diffusion on Mueller-Hinton agar was used to confirm the MDR status of each one, read according to CLSI recommendations [11]. The panel consisted of 12 antibiotics from the major classes: amoxicillin, amoxicillin-clavulanate, ceftriaxone, cefotaxime, ciprofloxacin, levofloxacin, gentamicin, amikacin, trimethoprim-sulfamethoxazole, imipenem, meropenem and nitrofurantoin. An isolate was defined as MDR if it was resistant to 3 or more types of antibiotics [12].

Acid Tolerance Assay

Acid tolerance was determined by the approach of Reuben et al. [8] with some modifications. Overnight MRS broth cultures were pelleted by centrifugation (6000 ×g, 10 min, 4°C), washed twice in sterile phosphate buffered saline (PBS, pH 7.2) and adjusted to around 10^9 CFU/mL. Each suspension (1 mL) was added to 9 mL of MRS broth adjusted to pH 2.0 or 3.0 with 1 N HCl, while control tubes were kept at pH 6.5. Tubes were kept at 37 °C and viable counts were determined at 0 and 3 h by the pour-plate method on MRS agar. The survival rate was computed as follows; Survival (%) = $\log \text{CFU/mL at 3 h} / \log \text{CFU/mL at 0 h} \times 100$.

Bile Salt Tolerance Assay

Bile tolerance was monitored as described by Sakandar et al [7]. Washed cells were inoculated at 1%v/v in MRS broth with 0.3 or 0.5% (w/v) oxgall (Sigma-Aldrich, USA) with bile free MRS broth as control. Viable counts were performed after 4 h at 37°C and survival was calculated as for the acid assay. All experiments were performed in triplicate and findings are expressed as mean ± standard deviation.

Preparation of Cell-Free Supernatant

Lactobacillus isolates were cultivated anaerobically in MRS broth at 37°C for 24 h. The culture was spun at 10,000 ×g for 15 min at 4°C and the supernatant taken off. To prevent interference of organic acids and hydrogen peroxide, the supernatant was adjusted to pH 6.5 with 1 N NaOH and then treated with catalase (1 mg/mL, Sigma-Aldrich) for 30 min at 37°C. The modified CFS was filtered through 0.22 µm Millipore filters and kept at -20°C until use [13].

Agar Well Diffusion Assay

Antagonistic activity was determined by agar well diffusion. Each target pathogen was adjusted to a 0.5 McFarland standard (about 1.5×10^8 CFU/mL) and equally swabbed on Mueller-Hinton agar. Wells of 6 mm diameter were cut with a sterile cork borer and

filled with 100 μL of the catalase-treated CFS that had been neutralised. The plates were incubated at 4°C for 2 h to allow diffusion of the CFS and then incubated at 37°C for 18 to 24 h. The inhibition zone sizes were expressed in mm as the mean of three replicates [14].

Partial Purification of Bacteriocin

Partial purification of bacteriocin of *L. plantarum* LP-7 was performed from its CFS by ammonium sulphate precipitation. Solid ammonium sulphate was added slowly to 500 mL CFS at 4°C with steady stirring until 70% saturation, and the mixture was left at 4°C overnight. The precipitate was pelleted (12,000 $\times g$, 30 min, 4°C), resuspended in a small volume of 20 mM sodium phosphate buffer (pH 6.0) and dialysed against the same buffer using a 3.5-kDa cut-off membrane (Spectrum Labs) for 24 h with three buffer changes. Protein was quantified by the Bradford technique, using bovine serum albumin as the standard [15].

Determination of Minimum Inhibitory Concentration

MIC values of the partly purified bacteriocin were determined by broth microdilution in 96-well plates. Two-fold dilutions from 6.25 to 400 $\mu\text{g/mL}$ were made in Mueller-Hinton broth, and each well received a standardized pathogen suspension (5×10^5 CFU/mL). Plates were read after 18 to 20 h at 37°C. The MIC was the lowest concentration with no visible growth; turbidity was checked by absorbance at 600 nm in a microplate reader. Positive (broth + inoculum) and negative (broth only) controls were included in every assay, and all assays were run in triplicate [16].

SDS-PAGE Analysis

The size of the partly purified bacteriocin was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Laemmli method, on a 15% resolving gel with a 5% stacking gel. A protein marker covering 10 to 250 kDa (Bio-Rad, USA) ran alongside the samples. Gels ran at 100 V for about 90 min, were stained with Coomassie Brilliant Blue R-250, and were destained in methanol, acetic acid, and water (40:10:50, v/v/v). Molecular mass was read off a standard curve built from the marker band positions [17].

Effect of Temperature and pH on Bacteriocin Activity

The thermal stability of the partially purified bacteriocin was determined by heating aliquots for 30 min at 40, 60, 80 and 100°C and for 15 min at 121°C (autoclaving). Samples were adjusted to each entire pH unit from 2.0 to 10.0 with HCl or NaOH and incubated at 37°C for 2 h for pH stability. The residual activity after each treatment was determined against *E. coli* MDR by agar well diffusion and expressed as a percentage of the untreated control [5].

Statistical Analysis

Every experiment was done in triplicate, and results are reported as mean $\pm$ SD. Data were analyzed in SPSS version 26.0 (IBM Corp., Armonk, NY, USA) by

one-way analysis of variance (ANOVA) with Tukey's HSD post hoc test for comparisons between groups. Differences were taken as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Isolation and Identification of *Lactobacillus* spp.

From the 60 samples, 45 probable *Lactobacillus* isolates were obtained on MRS agar; 32 from fermented dairy products and 13 from vaginal swabs. All these were Gram-positive, catalase-negative, oxidase-negative, non-motile rods, which is in accordance with the usual description of the genus [4]. The typical appearance of colonies of the five sample isolates on MRS agar after 48 h anaerobic incubation is shown in Figure 1. The colonies were white to creamy, convex and with smooth edges. Colony size ranged from 1 to 3 mm and was species-dependent; colonies of *L. plantarum* LP-7 were distinctly larger than those of the other four.

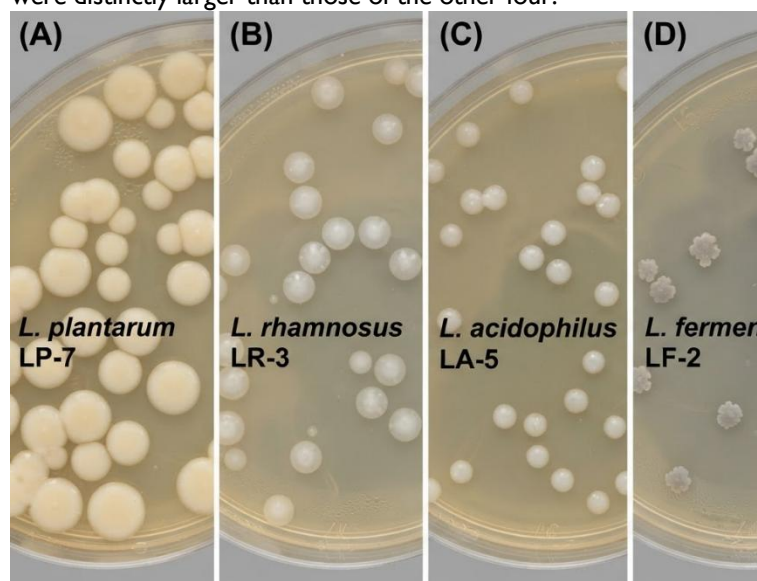


Figure 01: Colony morphology of representative *Lactobacillus* isolates on MRS agar after 48 h anaerobic incubation at 37°C. (A) *L. plantarum* LP-7, (B) *L. rhamnosus* LR-3, (C) *L. acidophilus* LA-5, (D) *L. fermentum* LF-2, (E) *L. casei* LC-4.

All isolates Gram-positive with oil immersion and were purple rods (Figure 2). The species did not look alike under the microscope. *L. plantarum* LP-7 rods were longer and slightly wider. *L. fermentum* LF-2 rods were shorter and sometimes slightly bent. *L. acidophilus* LA-5 developed long, slender rods, consistent with the classical description of this species [18].

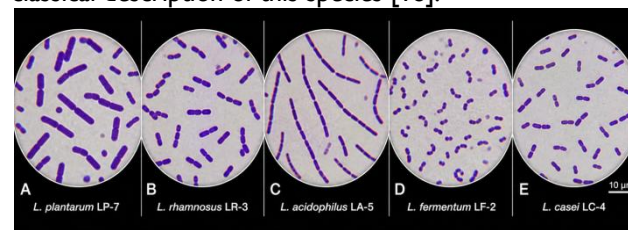


Figure 02: Representative *Lactobacillus* isolates after Gram staining, viewed under oil immersion ($\times 1000$).

All isolates appeared as Gram-positive rods. (A) *L. plantarum* LP-7, (B) *L. rhamnosus* LR-3, (C) *L. acidophilus* LA-5, (D) *L. fermentum* LF-2, (E) *L. casei* LC-4. Molecular identification by 16S rRNA gene sequencing assigned the isolates to five species: *L. plantarum* (n=14), *L. rhamnosus* (n=10), *L. acidophilus* (n=9), *L. fermentum* (n=7), and *L. casei* (n=5). The 16S rRNA amplicons of each representative isolate were run on an agarose gel and visualised under UV illumination, which revealed a single brilliant band of ~1500 bp (Figure 3). The dairy isolates were dominated by *L. plantarum*. This is in agreement with numerous previous assessments which mention it as one of the LAB most frequently isolated from traditional fermented foods in different parts of the world [18]. In contrast, the vaginal isolates were mainly *L. rhamnosus* and *L. acidophilus*, two species that are normally common in a healthy vaginal microbiome [19]. The distribution and identification results are given in Table I.

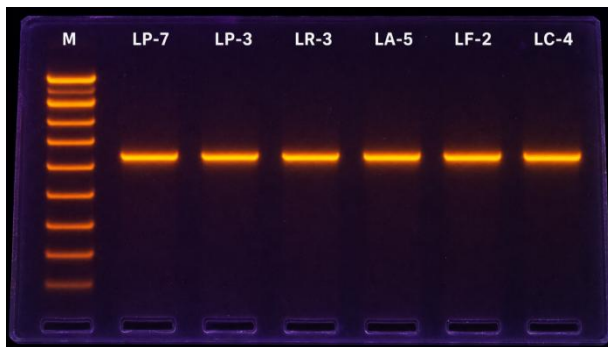


Figure 03: 16S rRNA gene PCR products on an agarose gel under UV transillumination. Lane M: DNA molecular weight marker; Lanes LP-7 through LC-4: amplicons (~1500 bp) from representative *Lactobacillus* isolates.

Table 01: Source distribution and molecular identification of the *Lactobacillus* isolates.

Species	Dairy (n)	Vaginal (n)	Total (n)	GenBank Acc.
<i>L. plantarum</i>	11	3	14	OQ987123
<i>L. rhamnosus</i>	5	5	10	OQ987124
<i>L. acidophilus</i>	6	3	9	OQ987125
<i>L. fermentum</i>	5	2	7	OQ987126
<i>L. casei</i>	5	0	5	OQ987127
Total	32	13	45	—

Acid Tolerance

Any organism that is to be used as an oral probiotic has to survive the acidity of the stomach first. Fig. 4 shows the results of 3 h at pH 2.0. All five typical isolates were viable however the survival rates varied a lot. The best strain was *L. plantarum* LP-7 at pH 2.0 with retention of 87.2 ± 2.4 % of the initial count and

94.5 ± 1.8 % at pH 3.0. The worst strain was *L. fermentum* LF-2 (58.1 ± 3.1 % and 75.4 ± 2.7 %, respectively) and the difference between the two strains was significant ($P < 0.05$). A similar trend was seen by Reuben et al. [8]: *L. plantarum* strains isolated from Nigerian traditional fermented foods were among the most acid-tolerant LAB in their collection, with many remaining above 80% viable at pH 2.0 for 3 h. This is partly due to the proton translocating F₀F₁-ATPase of *L. plantarum* and partly due to its ability to alter the fatty acid composition of its membrane at lower pH [20].

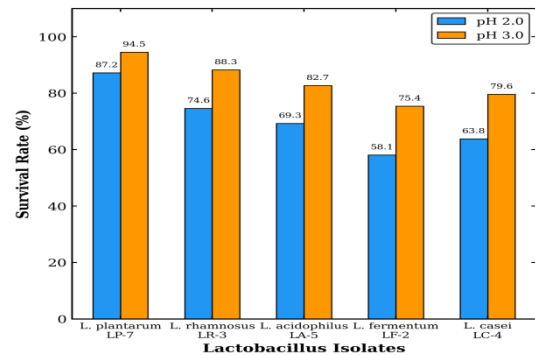


Figure 04: Survival (%) of the selected *Lactobacillus* isolates after 3 h at pH 2.0 and pH 3.0. Values are mean $\pm$ SD ($n = 3$).

Bile Salt Tolerance

Bile is the second hurdle. Bile salts damage the bacterial membrane and interfere with metabolism, so tolerance of physiological bile concentrations is a core probiotic criterion [7]. Figure 5 shows that every isolate survived in bile-supplemented broth, with viability falling as the oxgall concentration rose. Once again, *L. plantarum* LP-7 exhibited the strongest bile tolerance, with survival rates of 82.5 ± 2.1 % and 71.3 ± 2.8 % at 0.3% and 0.5% oxgall, respectively. *L. fermentum* LF-2 came last (61.4 ± 3.5 % and 48.9 ± 3.2 % at the same concentrations). Reuben et al. [8] likewise found that *L. plantarum* strains generally outperformed other lactobacilli in bile tolerance assays. A likely reason is bile salt hydrolase (BSH), an enzyme common in *L. plantarum*. BSH deconjugates bile acids, and the deconjugated forms are less toxic to the bacterial cell, which gives a plausible mechanism for the tolerance seen here [21].

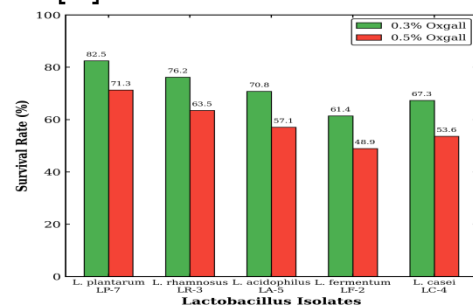


Figure 05: Survival (%) of the selected *Lactobacillus* isolates after 4 h in 0.3% and 0.5% oxgall. Values are mean $\pm$ SD ($n = 3$).

Antibiotic Susceptibility of Target MDR Pathogens

Each of the six clinical isolates resisted at least three antibiotic classes, which confirms their MDR status; Table 2 lists the profiles. *E. coli* resisted 9 of the 12 antibiotics tested and *K. pneumoniae* resisted 10, patterns typical of the ESBL-producing phenotypes reported with growing frequency from hospitals worldwide [2]. *P. aeruginosa* was resistant to the carbapenems imipenem and meropenem as well, which leaves very little to treat it with and is worrying from a clinical standpoint. Figure 6 illustrates representative antibiotic disc diffusion plates for three of the MDR isolates, clearly showing the absence of inhibition zones around most antibiotic discs (resistant) with a few sensitive discs showing clear halos.

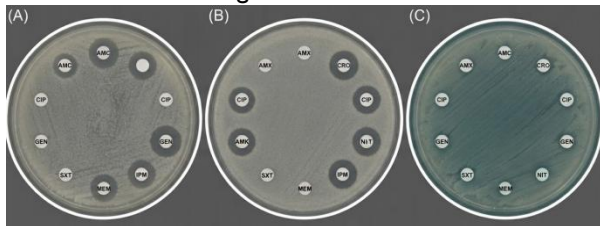


Figure 06: Antibiotic disc diffusion plates (Mueller-Hinton agar) for three of the MDR isolates: (A) *E. coli*, (B) *K. pneumoniae*, (C) *P. aeruginosa*. A clear zone around a disc means susceptibility; no zone means resistance.

Table 02: Antibiotic resistance profiles of the six target MDR clinical isolates.

Pathogen	Resistant to	No. of classes
<i>E. coli</i>	AMX, AMC, CRO, CTX, CIP, SXT, GEN, LEV, NIT	6
<i>K. pneumoniae</i>	AMX, AMC, CRO, CTX, CIP, SXT, GEN, AMK, LEV, NIT	7
<i>P. mirabilis</i>	AMX, AMC, CRO, CTX, CIP, SXT, GEN	5
<i>P. aeruginosa</i>	AMX, AMC, CRO, CTX, CIP, GEN, IPM, MEM	5
<i>S. typhimurium</i>	AMX, CRO, CTX, CIP, SXT, GEN, LEV	5
<i>S. flexneri</i>	AMX, AMC, CRO, CIP, SXT, GEN	4

AMX: amoxicillin; AMC: amoxicillin-clavulanate; CRO: ceftriaxone; CTX: cefotaxime; CIP: ciprofloxacin; LEV: levofloxacin; GEN: gentamicin; AMK: amikacin; SXT: trimethoprim-sulfamethoxazole; IPM: imipenem; MEM: meropenem; NIT: nitrofurantoin.

Antagonistic Activity of Cell-Free Supernatant

Neutralized, catalase-treated CFS from twelve *Lactobacillus* isolates was tested for antagonistic activity against the six MDR pathogens. Three isolates gave consistently large zones against every pathogen: *L. plantarum* LP-7, *L. rhamnosus* LR-3, and *L. acidophilus* LA-5. Figure 7 shows agar well diffusion plates for the

CFS of *L. plantarum* LP-7 against each of the six MDR targets, with the zone diameters written on the images.

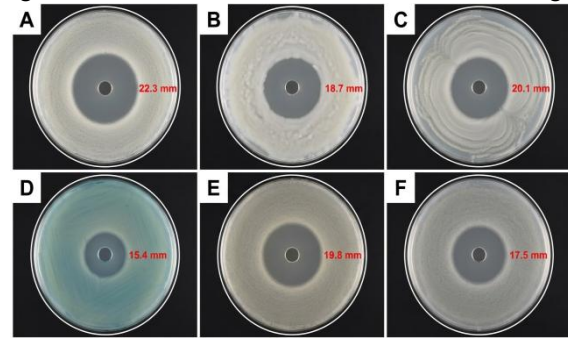


Figure 07: Inhibition zones produced by CFS of *L. plantarum* LP-7 in the agar well diffusion assay against six MDR pathogens: (A) *E. coli*, (B) *K. pneumoniae*, (C) *P. mirabilis*, (D) *P. aeruginosa*, (E) *S. typhimurium*, (F) *S. flexneri*. Zone diameters (mm) are given in red.

Against all six targets, the CFS of *L. plantarum* LP-7 gave the widest zones. The largest was against *E. coli* (22.3 ± 0.9 mm) and the smallest against *P. aeruginosa* (15.4 ± 1.1 mm). The *P. aeruginosa* result is no surprise. Its outer membrane lets very little through, and it carries several efflux pumps, so antimicrobial peptides in general work less well against it [14]. The strong effect on *E. coli* matters clinically, because *E. coli* causes most community-acquired and healthcare-associated UTIs [1]. Figure 8 compares the zone diameters of the three most active isolates in a bar chart, side by side for each pathogen.

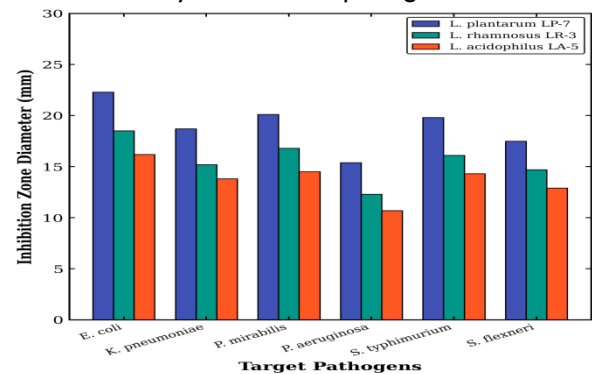


Figure 08: Inhibition zone diameters (mm) of neutralized CFS from the three most active *Lactobacillus* isolates against six MDR pathogens. Values are mean $\pm$ SD ($n = 3$).

The CFS had been neutralized and treated with catalase before testing, yet it still inhibited all six pathogens. Organic acids and hydrogen peroxide can therefore be ruled out as the main cause, and the effect is best explained by bacteriocin-like substances. Khochamit et al. [13] reached the same conclusion for bacterial culture supernatants whose proteinaceous antimicrobials stayed active after comparable pH adjustment and catalase treatment. Table 3 gives the full dataset for the twelve screened isolates.

Table 03: Inhibition zones (mm) produced by CFS of selected *Lactobacillus* isolates against the MDR pathogens.

Isolate	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>	<i>S. flexneri</i>
LP-7	22.3 ±0.9	18.7 ±1.2	20.1 ±0.8	15.4 ±1.1	19.8 ±1.0	17.5 ±0.7
LR-3	18.5 ±1.1	15.2 ±0.9	16.8 ±1.3	12.3 ±1.0	16.1 ±0.8	14.7 ±1.2
LA-5	16.2 ±0.8	13.8 ±1.1	14.5 ±0.7	10.7 ±0.9	14.3 ±1.0	12.9 ±1.3
LF-2	13.6 ±1.2	11.4 ±0.8	12.1 ±1.0	8.5 ±1.3	11.8 ±0.7	10.3 ±1.1
LC-4	14.8 ±0.9	12.5 ±1.0	13.2 ±1.2	9.4 ±0.8	12.9 ±0.9	11.6 ±1.0
LP-3	19.4 ±1.0	16.1 ±0.7	17.3 ±1.1	13.2 ±0.9	17.0 ±1.2	15.1 ±0.8
LP-11	17.8 ±1.3	14.6 ±1.0	15.9 ±0.9	12.0 ±1.2	15.4 ±0.8	13.8 ±1.1
LR-6	15.1 ±0.7	12.8 ±1.1	13.7 ±0.8	10.1 ±1.0	13.5 ±1.3	11.9 ±0.7
LA-2	14.5 ±1.0	12.1 ±0.9	12.8 ±1.1	9.2 ±0.7	12.4 ±1.0	10.8 ±1.2
LA-8	15.7 ±0.8	13.3 ±1.2	14.0 ±0.9	10.5 ±1.1	13.9 ±0.7	12.2 ±1.0
LF-5	12.3 ±1.1	10.2 ±0.8	11.0 ±1.0	7.8 ±1.2	10.5 ±0.9	9.4 ±1.3
LC-1	13.1 ±0.9	11.0 ±1.0	11.7 ±0.7	8.1 ±1.1	11.3 ±1.2	10.0 ±0.8

Partial Purification and SDS-PAGE of Bacteriocin

L. plantarum LP-7 had the best acid and bile tolerance and the strongest antagonistic activity, so we chose it for bacteriocin purification. Precipitation with ammonium sulfate at 70% saturation recovered about 68% of the activity present in the crude CFS, with a purification fold of 3.2 and a specific activity of 1280 AU/mg protein. On SDS-PAGE, the dialyzed fraction gave one clear band at about 6.5 kDa (Figure 9). That size falls within the range usually reported for the Class II bacteriocins of *L. plantarum* [5]. Plantaricins, the best-studied bacteriocins of this species, are mostly 3 to 10 kDa and act by forming pores in sensitive cells [15]. The gel also shows the purification step by step across the lanes. The crude CFS lane has many bands; the 70% ammonium sulfate fraction has one dominant low-molecular-weight band with fewer contaminants; and the dialyzed preparation has a single strong band at the target size. A bacteriocin of the same size, 6.5 kDa, has been reported from *Lactobacillus acidophilus* MSI [22].

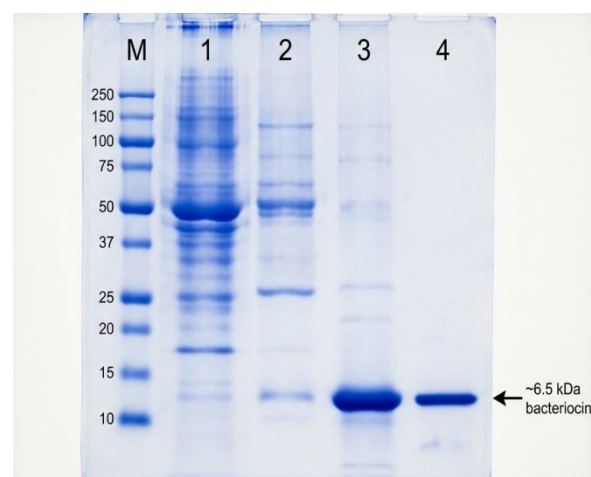


Figure 09: SDS-PAGE (15% gel) of the *L. plantarum* LP-7 bacteriocin at successive purification stages. Lane M: protein molecular weight marker; Lane 2: crude CFS; Lane 3: 40% ammonium sulfate fraction; Lane 4: 70% ammonium sulfate fraction; Lane 5: dialyzed bacteriocin. Arrow indicates the bacteriocin band at ~6.5 kDa.

Minimum Inhibitory Concentration of Bacteriocin

Figure 10 and Table 04 give the MIC values of the partly purified *L. plantarum* LP-7 bacteriocin against the six MDR pathogens. The lowest MIC was observed against *S. typhimurium* (25 µg/mL), followed by *E. coli* and *P. mirabilis* (both 50 µg/mL), *K. pneumoniae* and *S. flexneri* (both 100 µg/mL), and *P. aeruginosa* (200 µg/mL). These values are broadly comparable to those reported by Javadi et al. [16], who found MIC values of 30 to 120 µg/mL for a bacteriocin of *L. plantarum* against MDR *Acinetobacter baumannii*. The relatively higher MIC against *P. aeruginosa* is expected, as the intrinsic resistance mechanisms of this organism, particularly its restrictive outer membrane porins and constitutive efflux systems, tend to limit the access and activity of cationic antimicrobial peptides [10]. Nonetheless, an MIC of 200 µg/mL against a carbapenem-resistant *P. aeruginosa* isolate is still meaningful, especially considering that the bacteriocin was only partially purified and further optimization could potentially lower the effective concentration.

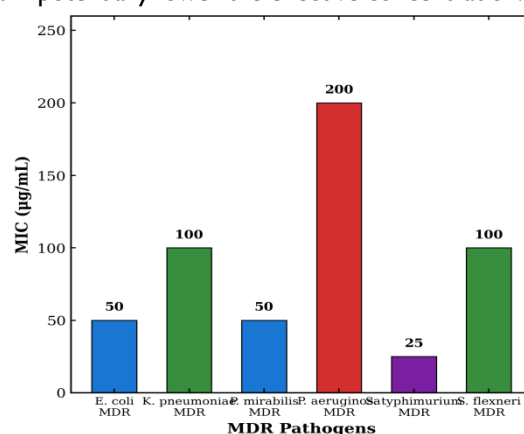


Figure 10: Minimum inhibitory concentration (MIC, $\mu\text{g/mL}$) of partially purified bacteriocin from *L. plantarum* LP-7 against six MDR pathogens.

Table 04: MIC of the partially purified *L. plantarum* LP-7 bacteriocin against the MDR pathogens.

Pathogen	Source	MIC ($\mu\text{g/mL}$)
<i>E. coli</i> MDR	Urine	50
<i>K. pneumoniae</i> MDR	Urine	100
<i>P. mirabilis</i> MDR	Urine	50
<i>P. aeruginosa</i> MDR	Urine	200
<i>S. typhimurium</i> MDR	Stool	25
<i>S. flexneri</i> MDR	Stool	100

Thermal and pH Stability of Bacteriocin

The effectiveness of a bacteriocin depends on its resistance to heat and pH changes. Figure 11 shows the residual activity of the partly purified LP-7 bacteriocin after each treatment. The peptide shows a fair degree of thermostability as it retained over 97% of activity after heating at 60°C for 30 min and about 89.6% when heated at 80°C . Losses increased at 100°C (72.4%) and after autoclaving at 121°C (41.8%). This is often the case for small, compact peptides. A near equivalent is Plantaricin LP 21-2 of *Lactobacillus plantarum* SHY 21-2 which still has ~ 96% of its activity after 15 min at 121°C [23]. The bacteriocin was most active in the pH range of moderately acidic to neutral, pH 4–7, the maximum activity being at pH 5.0 (98.7%). The activity dropped to 62.5% at pH 2.0 and 43.8% at pH 10.0 at the extremes, perhaps due to the denaturing of the peptide or the change in charge distribution under these conditions [5]. The physiological pH of the urinary tract and of the gastrointestinal environment both fall into the range in which the bacteriocin remained active, a point in favour of its possible clinical and biotechnological usage.

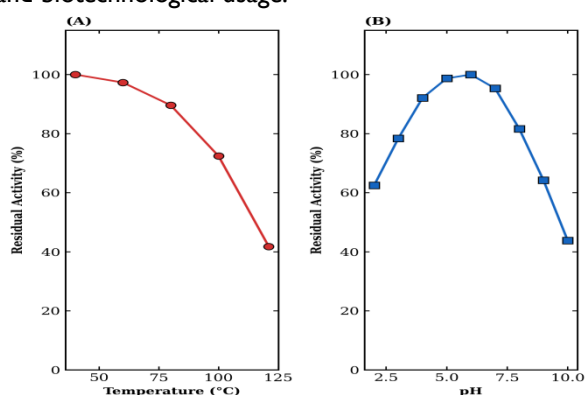


Figure 11: Residual activity (%) of the partially purified *L. plantarum* LP-7 bacteriocin against *E. coli* MDR after (A) heat treatment and (B) pH adjustment. Values are mean $\pm$ SD ($n = 3$).

CONCLUSION

Fermented dairy products from local sources, and the vaginal microbiome of healthy women, were both found to be good sources of varied *Lactobacillus* strains with clear probiotic and antibacterial potential. *L. plantarum* LP-7 was the dominant of the forty-five isolates in five species. It tolerated acid and bile better than the rest, and its CFS produced the biggest inhibition zones against all six MDR uropathogens and enteropathogens. Its partially pure bacteriocin, approximately 6.5 kDa on SDS-PAGE, inhibited all pathogens tested, with a MIC as low as 25 $\mu\text{g/mL}$ against *S. typhimurium*, and remained active over the range of temperatures and pH values found in the human body. On the basis of this information, LP-7 and its bacteriocin merit investigation as a bio-based alternative to antibiotics for infections caused by resistant organisms. Before this may be achieved, many steps need to be taken: the complete structure of the bacteriocin needs to be elucidated, its cytotoxicity toward mammalian cells needs to be investigated and its efficacy needs to be demonstrated in vivo in animal models of urinary tract and gastrointestinal infection.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

INFORMED CONSENT

Informed verbal consent was obtained from all female participants who provided vaginal swab specimens during routine gynecological examinations. The clinical bacterial isolates were obtained from routine diagnostic specimens with no additional patient intervention required.

ETHICAL STATEMENT

This study was approved by the Institutional Ethics Committee of the General Directorate of Al-Qadisiyah Education, Diwaniyah, Iraq. All experimental procedures involving human-derived samples were conducted in accordance with the ethical principles of the Declaration of Helsinki.

AUTHOR CONTRIBUTION

Lamyaa Sabeeh Abdullah (sole author): Conceptualization, study design, sample collection, laboratory investigation, data acquisition, formal analysis, statistical analysis, writing – original draft

preparation, writing – review and editing, and final approval of the manuscript.

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