EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2026, 27(7), 151–159
https://doi.org/10.12912/27197050/222450
ISSN 2719–7050, License CC-BY 4.0

Received: 2026.05.17
Accepted: 2026.06.15
Published: 2026.07.01

Environmental *Enterococcus* spp. in aquatic and terrestrial matrices from northwestern Algeria: Antimicrobial resistance profiling and preliminary *in vitro* evaluation of *Lactobacillus*-derived antagonism

Saliha Larouci[1,2*], Meriem Abdi[1], Sofiane Mohamed El Amine Benghali[3,4], Farid Bensalah[2]

[1] Laboratoire de Génétique Moléculaire et Cellulaire, Faculté des Sciences de la Nature et de la Vie, Université des Sciences et de la Technologie Mohamed Boudiaf (USTO-MB), BP 1505 El M'Naouar, 31000 Oran, Algeria
[2] Laboratoire de Génétique Microbienne, Département de Biologie, University Oran 1 Ahmed Ben Bella, BP 1524 El M'Naouar, 31000 Oran, Algeria
[3] Laboratoire Réseau de Surveillance Environnementale, Department of Biology, University of Oran 1 Ahmed Ben Bella, BP 1524 El M'Naouar, 31000 Oran, Algeria
[4] Département du Vivant et de l'Environnement, Faculté des Sciences de la Nature et de la Vie, Université des Sciences et de la Technologie Mohamed Boudiaf (USTO-MB), BP 1505 El M'Naouar, 31000 Oran, Algeria
* Corresponding author's e-mail: saliha.larouci@univ-usto.dz

ABSTRACT

Environmental enterococci are increasingly recognised as major reservoirs of antimicrobial resistance (AMR) determinants and reliable indicators of faecal contamination in aquatic and terrestrial ecosystems. This exploratory pilot study, conducted on a limited convenience sample from the Oran region (northwestern Algeria), pursued two interconnected objectives within a One Health framework: (i) to characterise the antimicrobial resistance profiles of environmental *Enterococcus* isolates recovered from hospital wastewater, municipal sewage sludge, manure-amended agricultural soil, and contaminated river water through species-specific *sodA*-targeted PCR; and (ii) to evaluate the preliminary in vitro antagonistic potential of four *Lactobacillus* spp. strains, confirmed at genus level by genus-specific PCR and pre-screened for safety, against multidrug-resistant (MDR) enterococcal isolates. A total of 13 isolates (8 *Enterococcus faecalis*, 5 *Enterococcus faecium*) were confirmed by species-specific PCR targeting the *sodA* gene. Universal ciprofloxacin resistance (100%) and high-level ampicillin resistance (75–80%) were recorded; the genetic basis of fluoroquinolone resistance was not characterised. All isolates remained fully susceptible to linezolid and tigecycline. One *E. faecalis* isolate from hospital wastewater displayed a phenotypic profile consistent with vancomycin-resistant *Enterococcus* (VRE); genotypic confirmation by vanA/vanB PCR was not performed within the scope of this study; this isolate is classified as putative-VRE throughout. The four *Lactobacillus* spp. strains (designated LBAS-01 to LBAS-04, confirmed at genus level by genus-specific PCR) were confirmed free of transferable antibiotic resistance genes and haemolytic activity. All produced measurable in vitro inhibitory activity against MDR enterococci (inhibition zone diameters: 9–20 mm); partial mechanistic characterisation indicated that two strains produce a proteinaceous inhibitory substance consistent with bacteriocin activity.

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, antimicrobial resistance, vancomycin-resistant enterococci, *sodA*-PCR, *Lactobacillus* spp., biocontrol, Algeria, One Health.

INTRODUCTION

The global rise of antimicrobial resistance (AMR) constitutes one of the most pressing threats to public and environmental health in the twenty-first century. The World Health Organization (WHO) projects that resistant infections could cause 10 million deaths annually by 2050 in

the absence of coordinated action (O'Neill, 2016). The environment, encompassing wastewater treatment systems, agricultural soils, and surface water bodies, is now recognised as an active reservoir of resistance determinants, facilitating selection and dissemination of resistant bacteria across ecological compartments (Berendonk et al., 2015).

Among environmentally and clinically relevant bacterial genera, *Enterococcus* spp. occupies a singular position. Originally considered commensal inhabitants of the gastrointestinal tract of humans and animals, enterococci have emerged as leading nosocomial pathogens and robust environmental survivors (Fisher and Phillips, 2009). Their capacity for horizontal gene transfer (HGT), mediated through conjugative plasmids (notably carrying Tn1546) and integrative conjugative elements (ICEs, including Tn916), renders them highly efficient vectors for the dissemination of resistance genes – including those conferring glycopeptide, aminoglycoside, and fluoroquinolone resistance – across the human-animal-environment interface (Werner et al., 2008).

Enterococcus faecalis and *Enterococcus faecium* are the two clinically and ecologically most important species. Their detection in hospital wastewater, municipal sewage, agricultural runoff, and surface waters establishes these matrices as critical hotspots for MDR strain emergence and dissemination (Guzman Prieto et al., 2016; Iversen et al., 2002). The One Health concept – recognising the inextricable interconnection between human, animal, and environmental health – provides the essential integrative framework for addressing AMR as a systemic, multi-compartment problem (WHO/FAO/OIE, 2019). In northern Algeria, epidemiological data on environmental AMR, particularly in enterococcal populations from diverse ecological matrices, remain scarce.

Accurate species-level identification of environmental enterococci is a prerequisite for effective surveillance. Molecular methods targeting the *sodA* gene have demonstrated high specificity and sensitivity for discriminating *E. faecalis* from *E. faecium* in complex environmental matrices (Jackson et al., 2004; Poyart et al., 2000), and are now the standard for molecular confirmation.

Concurrently, lactic acid bacteria (LAB), particularly *Lactobacillus* spp., have attracted growing interest as candidate biocontrol agents for application in contaminated environmental systems (Alakomi et al., 2000; Cotter et al., 2013). Their antagonistic activity against MDR pathogens – mediated through organic acid production, hydrogen peroxide generation, and bacteriocin secretion – represents a potentially sustainable strategy to mitigate AMR dissemination. However, before any applied biocontrol strategy can be considered, prerequisite conditions must be fulfilled: safety assessment of candidate strains, in vitro mechanistic characterisation, and environmental validation under ecologically relevant conditions. The present exploratory study was conducted using a limited convenience sample collected from four contrasting environmental matrices (hospital wastewater, municipal sewage sludge, agricultural soil, and river water) in the Oran region (Algeria). The aim of this study was to provide a preliminary One Health-oriented assessment of environmental *Enterococcus* spp. as reservoirs of antimicrobial resistance across contrasting ecological matrices in northern Algeria, and to evaluate the potential of *Lactobacillus* spp. as antagonistic biological agents against multidrug-resistant enterococci. We hypothesised that anthropogenically impacted environments, particularly hospital-associated effluents, act as reservoirs of multidrug-resistant enterococci, and that *Lactobacillus* spp. exhibit strain-dependent inhibitory activity against these isolates, potentially mediated by organic acids and bacteriocin-like compounds.

MATERIALS AND METHODS

Study sites and sample collection

Environmental samples were collected from four ecological matrices in the Oran region (35°41'N, 0°37'W; northwestern Algeria) during a single sampling campaign (October–November 2023): hospital wastewater (HW; n = 3 samples from the effluent outlet of a public hospital), municipal sewage sludge (SS; n = 3 samples from the dewatering stage of the Oran metropolitan wastewater treatment plant), manure-amended agricultural soil (AS; n = 4 samples from peri-urban plots receiving regular cattle manure applications), and contaminated river water (RW; n = 3 samples from the Oued Raz-El-Ain course downstream of urban discharge points). Samples were collected aseptically (1 L sterile containers for liquids; 500 g sterile bags for solids) and transported to the laboratory within 2 h under refrigerated conditions (4 °C). All samples were

processed within 24 h of collection. As this was a single-season sampling campaign, temporal variability was not assessed; this constitutes an acknowledged limitation.

Isolation and preliminary phenotypic characterisation

Enterococcal isolation was performed on Slanetz and Bartley selective agar (Oxoid, UK) supplemented with TTC (0.1 g L^{-1}). Water samples (100 mL) were filtered through 0.45 µm membranes (Millipore, USA). Soil and sludge matrices were serially diluted and spread-plated in duplicate. All plates were incubated at 37°C for 48 h. Isolates were confirmed as enterococci by Gram staining, catalase test (negative), bile–aesculin hydrolysis (positive), and growth in 6.5% NaCl at 37 °C. Isolate recovery per matrix was as follows: hospital wastewater (n = 3), municipal sewage sludge (n = 4), manure-amended agricultural soil (n = 3), and contaminated river water (n = 3), totalling 13 confirmed isolates. These figures represent phenotypically confirmed presumptive isolates submitted to molecular identification and do not constitute quantitative CFU abundance estimates. No CFU/g or CFU/mL data were collected; this constitutes an acknowledged limitation requiring quantitative follow-up in future studies.

Molecular identification by sodA-Targeted PCR

Genomic DNA was extracted from all 13 isolates using the Qiagen DNeasy Blood & Tissue Kit (QIAGEN GmbH, Hilden, Germany) with an additional lysozyme pre-treatment step (10 mg mL^{-1}, 37 °C, 60 min). DNA purity and concentration were assessed by NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); all extracts presented A260/A280 ratios of 1.80–1.95 and A260/A230 ratios ≥ 1.90. Species-level identification used *sodA*-targeted species-specific PCR according to Poyart et al. (2000): primer pair FL1 (5′-ATCAAGTACAGTTAGTCT-3′) / FL2 (5′-ACGATTCAAAGCTAACTG-3′) for *E. faecalis* (expected amplicon: 360 bp); EFM1 (5′-GAAAAAACAATAGAAGAATTAT-3′) / EFM2 (5′-TGCTTTTTTGAATTTCTTC-3′) for *E. faecium* (expected amplicon: 199 bp). Thermocycling conditions: initial denaturation 95 °C/5 min; 30 cycles of 95 °C/30 s, 56 °C/30 s, 72 °C/45 s; final extension 72 °C/10 min. All 13 isolates were run in a single gel session (2% agarose, EtBr 0.5 mg L^{-1}); *E. faecalis* ATCC 29212 and *E. faecium* ATCC 35667 served as positive controls; a no-template control (NTC) confirmed absence of contamination.

Antimicrobial susceptibility testing

Susceptibility was assessed by Kirby–Bauer disk diffusion on Mueller–Hinton agar (CLSI, 2022). Six antimicrobial agents were tested: ampicillin (AMP, 10 µg), vancomycin (VAN, 30 µg), teicoplanin (TEC, 30 µg), linezolid (LZD, 30 µg), tigecycline (TGC, 15 µg), and ciprofloxacin (CIP, 5 µg); all Bio-Rad, France. MDR was defined per Magiorakos et al. (2012). Resistance rates are expressed with 95% Wilson score confidence intervals (CI).

Minimum inhibitory concentration (MIC) determination and genotypic confirmation of glycopeptide resistance (vanA/vanB PCR) could not be performed within the scope of this exploratory study due to resource constraints. Phenotypic disk diffusion was the sole AST method employed. The putative-VRE classification of isolate EF-HW2 therefore rests on phenotypic criteria only and carries an inherent risk of false-positive classification (Woodford, 1998). MIC determination and vanA/vanB genotyping are identified as priorities for follow-up work. Similarly, genotypic characterisation of fluoroquinolone resistance (gyrA/parC QRDR sequencing; PMQR gene screening) was beyond the scope of this study.

Identification and safety assessment of *Lactobacillus* spp.

Genus-level identification by PCR

Four Lactobacillus spp. strains (LBAS-01 to LBAS-04), isolated from traditional Algerian fermented dairy products (lben and butter), were confirmed at genus level by genus-specific PCR using the primers V3-357f / V4-R806r targeting the V3–V4 hypervariable region of the 16S rRNA gene (expected amplicon: ~480 bp). *Lactobacillus delbrueckii* ATCC 11842 served as positive control; a no-template control confirmed absence of contamination. Species-level identification was not performed in the current study; all four strains are therefore designated *Lactobacillus* spp. LBAS-01 to LBAS-04 throughout. Species-level characterisation by 16S rRNA gene

full-length sequencing or multilocus sequence typing (MLST) is identified as a priority for future work.

Safety assessment

All four strains were screened for transferable antibiotic resistance genes and haemolytic activity prior to antagonism testing, in accordance with EFSA (2012) minimum safety criteria.

In vitro antagonism assay

Strains were pre-cultured in MRS broth (Oxoid, UK) at 37 °C for 18–24 h under anaerobic conditions. Cell-free culture supernatants (CFS) were obtained by centrifugation at 10.000 × g for 15 min at 4 °C and filtration through 0.22 µm membranes. Three CFS preparations were tested: (i) native CFS (pH 3.8–4.2); (ii) pH-neutralised CFS (pH 6.5 ± 0.1 with 1 mol L^{-1} NaOH); and (iii) proteinase K-treated CFS (100 µg mL^{-1}, 37 °C, 120 min, inactivated at 80 °C for 20 min). Disc diffusion assays were performed on MHA supplemented with 10% (v/v) MRS broth, seeded with MDR *Enterococcus* isolates at 0.5 McFarland. Sterile 6 mm discs were impregnated with 20 µL CFS, pre-diffused at 4 °C for 2 h, then incubated at 37 °C for 24 h. Inhibition zone diameters were measured with a digital calliper in triplicate independent assays. Each independent assay constituted a biological replicate; measurements within each assay were technical replicates (n = 3 per biological replicate). Activity was classified as: strong (≥ 16 mm), moderate (11–15 mm), or weak (< 11 mm; Tagg and McGiven, 1971). Results are expressed as mean ± SD. The following methodological limitations of the antagonism assay are acknowledged: (i) no positive antibiotic control was included on assay plates; (ii) the contribution of H_2O_2 to inhibitory activity was not quantified, as no catalase-treated CFS arm was included — this remains an unresolved mechanistic variable; (iii) supplementation of MHA with 10% MRS broth may influence agar diffusion kinetics relative to standard MHA, and absolute zone diameter values should be interpreted with this caveat.

Statistical analysis

Resistance rates are expressed as percentages with 95% Wilson score CI. Inhibition zone diameters are presented as mean ± SD of triplicate independent assays. Given the small group sizes (n = 4 zone values per species group for antagonism comparisons), no inferential between-group statistical comparisons were performed. All data are presented as descriptive summaries only. Analyses were performed in GraphPad Prism v9.0.

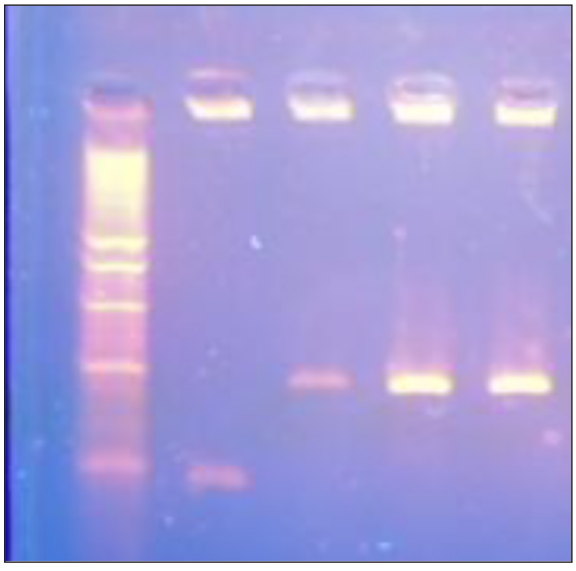

Figure 1. Agarose gel electrophoresis (2%) of sodA-targeted PCR products for Enterococcus species identification. Lane 1: 100 bp DNA ladder; Lane 2: *E. faecium* (199 bp, EFM1/EFM2 primers); Lane 3–5: *E. faecalis* (360 bp, FL1/FL2 primers)

RESULTS

Isolation and phenotypic characterisation

Thirteen presumptive *Enterococcus* isolates were recovered: 3 from hospital wastewater, 4 from sewage sludge, 3 from agricultural soil, and 3 from river water. All displayed genus-consistent morphology, negative catalase reaction, positive bile–aesculin hydrolysis, and growth in 6.5% NaCl at 37 °C.

Molecular identification by sodA-Targeted PCR

sodA-targeted PCR unambiguously identified all 13 isolates: 8 produced the 360 bp FL1/FL2 amplicon (*E. faecalis*); 5 produced the 199 bp EFM1/EFM2 amplicon (*E. faecium*). No non-specific amplification was detected. Positive controls produced expected amplicons; the NTC produced no band (Table 1, Figure 1).

Table 1. Distribution of confirmed Enterococcus isolates by species, sampling source, sodA amplicon size, and MDR status. HW: hospital wastewater; SS: sewage sludge; AS: agricultural soil; RW: river water. *Putative VRE phenotype (phenotypic co-resistance to VAN and TEC; vanA/vanB PCR confirmation pending). †MDR: non-susceptibility to ≥ 1 agent in ≥ 3 antimicrobial categories (Magiorakos et al., 2012)

Isolate code	Sampling source	Species confirmed	sodA amplicon (bp)	Matrix type	MDR†	Notes
EF-HW1	Hospital wastewater	*E. faecalis*	360	Clinical effluent	Yes	
*EF-HW2**	Hospital wastewater	*E. faecalis*	360	Clinical effluent	Yes	Putative VRE*
EF-SS1	Sewage sludge	*E. faecalis*	360	Municipal	Yes	
EF-SS2	Sewage sludge	*E. faecalis*	360	Municipal	No	CIP-R only
EF-AS1	Agricultural soil	*E. faecalis*	360	Agricultural	Yes	
EF-RW1	River water	*E. faecalis*	360	Aquatic	Yes	
EF-RW2	River water	*E. faecalis*	360	Aquatic	No	CIP-R only
EF-RW3	River water	*E. faecalis*	360	Aquatic	Yes	
EFM-HW1	Hospital wastewater	*E. faecium*	199	Clinical effluent	Yes	
EFM-SS1	Sewage sludge	*E. faecium*	199	Municipal	Yes	
EFM-AS1	Agricultural soil	*E. faecium*	199	Agricultural	Yes	
EFM-AS2	Agricultural soil	*E. faecium*	199	Agricultural	No	CIP-R only
EFM-RW1	River water	*E. faecium*	199	Aquatic	Yes	

Antimicrobial susceptibility profiles

Enterococcus faecalis (n = 8): Universal ciprofloxacin resistance (100%; 95% CI: 67.6–100%) and predominant ampicillin resistance (75%; 6/8; 95% CI: 40.9–92.9%) were recorded. Linezolid and tigecycline susceptibility was fully retained. Isolate EF-HW2, recovered from hospital wastewater, displayed co-resistance to vancomycin and teicoplanin on disk diffusion, consistent with putative VRE classification (phenotypic criteria only; genotypic confirmation not performed. Enterococcus faecium (n = 5): Total ciprofloxacin resistance (100%; 95% CI: 56.6–100%) and ampicillin resistance (80%; 4/5; 95% CI: 37.4–96.4%) were recorded. All five isolates remained fully susceptible to VAN, TEC, LZD, and TGC (Figures 2, 3, Tables 2, 3).

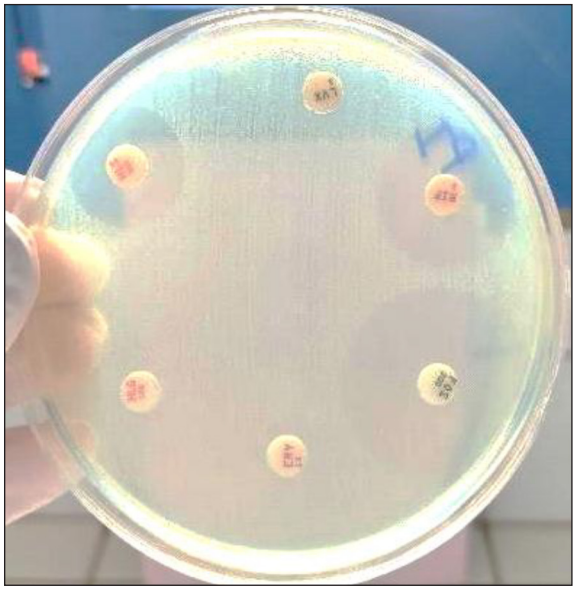

Figure 2. Disc diffusion antagonism assay showing inhibition zones produced by *Lactobacillus* spp. cell-free supernatants (CFS) against *Enterococcus faecalis* (MDR isolates) on MRS-MHA agar after 24 h at 37 °C. Clear zones indicate inhibitory activity

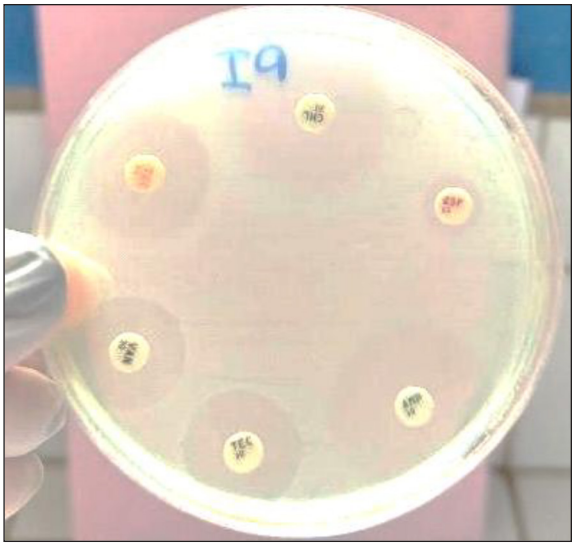

Figure 3. Disc diffusion antagonism assay showing inhibition zones produced by *Lactobacillus* spp. cell-free supernatants (CFS) against *Enterococcus faecium* (MDR isolates) on MRS-MHA agar after 24 h at 37 °C

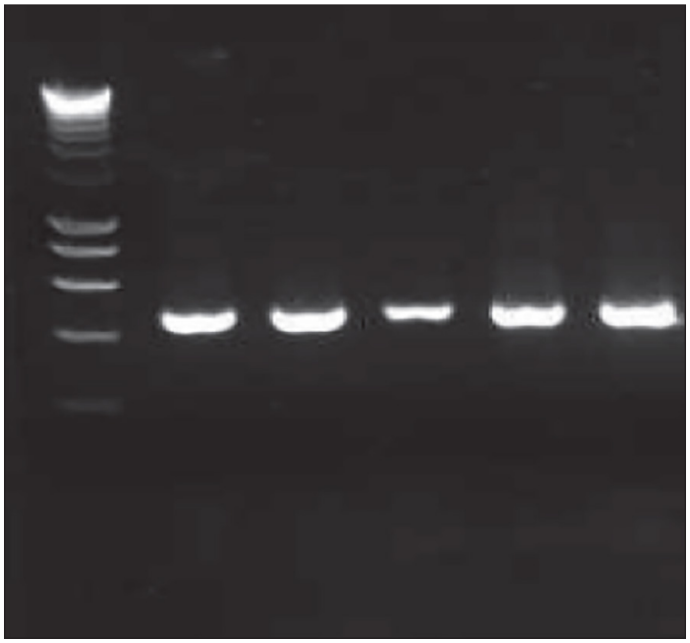

Figure 4. Agarose gel electrophoresis (0.8%) of 16S rRNA gene PCR products (480 bp) for Lactobacillus genus identification. M – 200 bp DNA size marker (Smart ladder); Lane 1 – positive control (Lb. delbruckii ATCC 11842); Lanes 2–6 – *Lactobacillus* spp. isolates LBAS-01 to LBAS-04

Table 2. Individual antimicrobial susceptibility profiles of all 13 isolates: AMP – ampicillin, VAN – vancomycin, TEC – teicoplanin, LZD – linezolid, TGC – tigecycline, CIP – ciprofloxacin, R – resistant, S – susceptible, putative VRE – vanA/vanB PCR confirmation pending), MDR – non-susceptibility to ≥ 1 agent in ≥ 3 antimicrobial categories

Isolate	Species	AMP	VAN	TEC	LZD	TGC	CIP	MDR
EF-HW1	*E. faecalis*	R	S	S	S	S	R	Yes
*EF-HW2**	*E. faecalis*	R	R	R	S	S	R	Yes – putative VRE
EF-SS1	*E. faecalis*	R	S	S	S	S	R	Yes
EF-SS2	*E. faecalis*	S	S	S	S	S	R	No
EF-AS1	*E. faecalis*	R	S	S	S	S	R	Yes
EF-RW1	*E. faecalis*	R	S	S	S	S	R	Yes
EF-RW2	*E. faecalis*	S	S	S	S	S	R	No
EF-RW3	*E. faecalis*	R	S	S	S	S	R	Yes
EFM-HW1	*E. faecium*	R	S	S	S	S	R	Yes
EFM-SS1	*E. faecium*	R	S	S	S	S	R	Yes
EFM-AS1	*E. faecium*	R	S	S	S	S	R	Yes
EFM-AS2	*E. faecium*	S	S	S	S	S	R	No
EFM-RW1	*E. faecium*	R	S	S	S	S	R	Yes

Table 3. Comparative resistance rates (%) with 95% Wilson score confidence intervals (CI) by species; putative VRE phenotype (EF-HW2); phenotypic classification only, genotypic confirmation pending

Antibiotic	*E. faecalis* (n = 8)	95% CI	*E. faecium* (n = 5)	95% CI
Ampicillin (AMP)	75% (6/8)	40.9–92.9%	80% (4/5)	37.4–96.4%
Vancomycin (VAN)	12.5% (1/8)*	2.2–47.1%	0% (0/5)	0–43.4%
Teicoplanin (TEC)	12.5% (1/8)*	2.2–47.1%	0% (0/5)	0–43.4%
Linezolid (LZD)	0% (0/8)	0–37.0%	0% (0/5)	0–43.4%
Tigecycline (TGC)	0% (0/8)	0–37.0%	0% (0/5)	0–43.4%
Ciprofloxacin (CIP)	100% (8/8)	67.6–100%	100% (5/5)	56.6–100%

Identification of *Lactobacillus* spp.

Genus-specific PCR confirmed all four strains as belonging to the genus *Lactobacillus*; they are designated LBAS-01 to LBAS-04 throughout. Species-level identification was not performed in the current study. The expected ~480 bp amplicon was obtained for all four strains. Positive control (*L. delbrueckii* ATCC 11842) and NTC produced expected results (Figure 4).

Antagonistic activity of *Lactobacillus* spp. against MDR enterococci

All four *Lactobacillus* spp. strains demonstrated measurable inhibitory activity against MDR *E. faecalis* and *E. faecium* using native CFS (Table 4). LBAS-02 produced the strongest activity (18 ± 1.1 mm vs. *E. faecalis*; 20 ± 0.9 mm vs. *E. faecium*; strong inhibition), followed by LBAS-04 (16 ± 0.8 mm and 17 ± 0.9 mm; strong). LBAS-03 produced moderate inhibition (14 ± 0.7 and 15 ± 1.0 mm). LBAS-01 yielded the weakest activity (9 ± 0.6 mm vs. *E. faecalis*; 12 ± 0.8 mm vs. *E. faecium*). Inhibitory activity of LBAS-02 and LBAS-04 was substantially reduced after proteinase K treatment, indicating a bacteriocin contribution. No inferential statistical comparisons between species groups were performed; data are presented descriptively only (Figure 5).

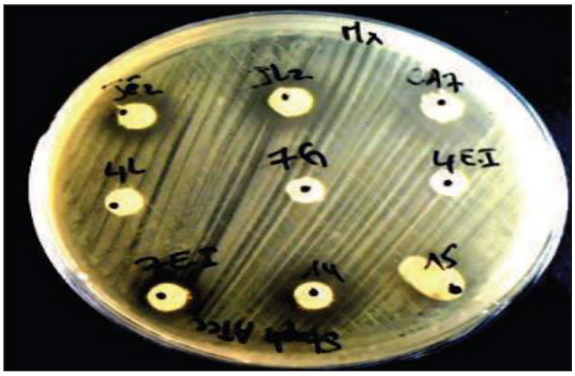

Figure 5. Disc diffusion antagonism assay showing inhibition zones produced by *Lactobacillus* spp. cell-free supernatants (CFS) against *E. faecalis* MDR isolate EF-HW2 on MHA supplemented with 10% MRS broth after 24 h at 37 °C. Labelled discs: L1 = LBAS-01; L2 = LBAS-02; L3 = LBAS-03; L4 = LBAS-04

DISCUSSION

The recovery of both *E. faecalis* and *E. faecium* from all four environmental matrices corroborates the ubiquitous distribution of enterococci in anthropogenically impacted environments (Fisher and Phillips, 2009). Hospital wastewater and sewage sludge represent primary entry points for clinically selected resistant strains into broader environmental compartments (Berendonk et al., 2015). The findings are strictly exploratory; the small convenience sample (n = 13 isolates) and single-season collection preclude any epidemiological generalisation. Universal ciprofloxacin resistance (100%) is the most ecologically significant resistance finding. The wide confidence intervals reflect the limited sample size. The genetic basis of fluoroquinolone resistance was not determined; chromosomal mutations in the QRDRs of *gyrA* and *parC* are the most prevalent mechanism, but PMQR genes represent a transferable risk of particular concern. Genotypic characterisation of fluoroquinolone resistance is recommended as a priority for follow-up studies.

The putative VRE phenotype of EF-HW2 from hospital wastewater is ecologically noteworthy. However, this classification rests solely on phenotypic disk diffusion, which carries a non-negligible false-positive risk (Woodford, 1998). MIC determination and vanA/vanB PCR confirmation could not be performed within the

Table 4. Inhibition zone diameters (mm ± SD; mean of three independent assays) produced by four Lactobacillus spp. strains against MDR Enterococcus isolates using three CFS preparations. Classification: strong ≥ 16 mm; moderate 11–15 mm; weak < 11 mm: pH-adj – pH-neutralised CFS (pH 6.5 ± 0.1); PK-treated – proteinase K-treated CFS (100 µg mL^{-1}); Ef – *E. faecalis*; Efm – *E. faecium*; PK – proteinase K

Strain	Species	Native vs. Ef (mm)	Classif.	pH-adj vs. Ef (mm)	Classif.	PK-treated vs. Ef (mm)	Classif.	Bacteriocin?
LBAS-01	*Lactobacillus spp.*	9 ± 0.6	Weak	9 ± 0.5	Weak	8 ± 0.7	Weak	Unlikely
LBAS-02	*Lactobacillus spp.*	18 ± 1.1	Strong	16 ± 1.0	Strong	7 ± 0.8	Weak	Likely
LBAS-03	*Lactobacillus spp.*	14 ± 0.7	Moderate	13 ± 0.8	Moderate	13 ± 0.9	Moderate	Unlikely
LBAS-04	*Lactobacillus spp.*	16 ± 0.8	Strong	14 ± 0.7	Moderate	8 ± 0.7	Weak	Likely

scope of this exploratory study due to resource constraints. The designation 'putative VRE' must therefore be interpreted with appropriate caution and should not be used to draw definitive conclusions about VRE prevalence in the study environment. Full susceptibility to linezolid and tigecycline indicates that last-resort compounds have not yet exerted significant selective pressure in these matrices, consistent with their restricted clinical use (Cattoir and Leclercq, 2013).

The in vitro inhibitory activity demonstrated by all four *Lactobacillus* spp. strains against MDR enterococci aligns with published data on LAB biocontrol potential (Cotter et al., 2013; Zommiti et al., 2020). The partial mechanistic characterisation provides preliminary evidence for bacteriocin-type inhibition by LBAS-02 and LBAS-04. However, several important limitations must be acknowledged: (i) species-level identification of the four Lactobacillus strains was not performed – they are confirmed at genus level only by genus-specific PCR; species characterisation by 16S rRNA gene sequencing or MLST is identified as a priority for follow-up work; (ii) H_2O_2 contribution to inhibitory activity was not quantified; (iii) no positive antibiotic control was included in the antagonism assay.

CONCLUSIONS

Environmental matrices from the Oran region harbour enterococcal isolates with clinically relevant antimicrobial resistance profiles, including universal fluoroquinolone resistance and a putative glycopeptide-resistance phenotype requiring genotypic confirmation before definitive VRE classification can be assigned. These findings underscore the role of hospital wastewater and municipal sewage as critical entry points for resistant bacteria into the broader environment.

Lactobacillus spp. strains (LBAS-01 to LBAS-04) isolated from traditional fermented dairy products demonstrated in vitro inhibitory activity against MDR enterococcal isolates. Preliminary mechanistic evidence indicates a bacteriocin contribution for two strains (LBAS-02 and LBAS-04). These results should be interpreted within the explicit limitations of this exploratory pilot study: small convenience sample, absence of genotypic AMR confirmation, genus-level-only identification of Lactobacillus strains, and unquantified H_2O_2 contribution in the antagonism assay. They nonetheless support advancement to the next tier of characterisation – including species-level identification, MIC determination, vanA/vanB genotyping, and ecologically representative mesocosm studies.

REFERENCES

1. Alakomi, H. L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., Helander, I. M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology, 66*(5), 2001–2005. https://doi.org/10.1128/AEM.66.5.2001-2005.2000
2. Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology, 13*(5), 310–317. https://doi.org/10.1038/nrmicro3439
3. Cattoir, V., Leclercq, R. (2013). Twenty-five years of shared life with vancomycin-resistant enterococci. *Journal of Antimicrobial Chemotherapy, 68*(4), 731–742. https://doi.org/10.1093/jac/dks469
4. Clinical and Laboratory Standards Institute (CLSI). (2022). *Performance standards for antimicrobial susceptibility testing (32nd ed.). CLSI supplement M100*. Clinical and Laboratory Standards Institute.
5. Cotter, P. D., Ross, R. P., Hill, C. (2013). Bacteriocins — a viable alternative to antibiotics? *Nature Reviews Microbiology, 11*(2), 95–105. https://doi.org/10.1038/nrmicro2937
6. European Food Safety Authority (EFSA). (2012). Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal, 10*(6), 2740. https://doi.org/10.2903/j.efsa.2012.2740
7. Fisher, K., Phillips, C. (2009). The ecology, epidemiology and virulence of Enterococcus. *Microbiology, 155*(6), 1749–1757. https://doi.org/10.1099/mic.0.026385-0
8. Guzman Prieto, A. M., van Schaik, W., Rogers, M. R. C., Coque, T. M., Baquero, F., Corander, J., Willems, R. J. L. (2016). Global emergence and dissemination of enterococci as nosocomial pathogens: Attack of the clones? *Frontiers in Microbiology, 7*, 788. https://doi.org/10.3389/fmicb.2016.00788
9. Iversen, A., Kuøhn, I., Franklin, A., Møllby, R. (2002). High prevalence of vancomycin-resistant enterococci in Swedish sewage. *Applied and Environmental Microbiology, 68*(6), 2838–2842. https://doi.org/10.1128/AEM.68.6.2838-2842.2002
10. Jackson, C. R., Fedorka-Cray, P. J., Barrett, J. B. (2004). Use of a genus- and species-specific multiplex PCR for identification of enterococci. *Journal of Clinical Microbiology, 42*(8), 3558–3565. https://doi.org/10.1128/JCM.42.8.3558-3565.2004

11. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x
12. Manaia, C. M., Macedo, G., Fatta-Kassinos, D., Nunes, O. C. (2018). Antibiotic resistance in urban aquatic environments: Can it be controlled? *Applied Microbiology and Biotechnology, 100*(4), 1543–1557. https://doi.org/10.1007/s00253-015-7202-0
13. O'Neill, J. (2016). Tackling drug-resistant infections globally: Final report and recommendations. Review on Antimicrobial Resistance. HM Government & Wellcome Trust.
14. Poyart, C., Quesnes, G., Trieu-Cuot, P. (2000). Sequencing the gene encoding manganese-dependent superoxide dismutase for rapid species identification of enterococci. *Journal of Clinical Microbiology, 38*(2), 660–666. https://doi.org/10.1128/JCM.38.2.660-666.2000
15. Tagg, J. R., McGiven, A. R. (1971). Assay system for bacteriocins. *Applied Microbiology, 21*(5), 943. https://doi.org/10.1128/am.21.5.943-943.1971
16. Werner, G., Coque, T. M., Hammerum, A. M., Hope, R., Hryniewicz, W., Johnson, A., Woodford, N. (2008). Emergence and spread of vancomycin resistance among enterococci in Europe. *Eurosurveillance, 13*(47), 19046. https://doi.org/10.2807/ese.13.47.19046-en
17. WHO/FAO/OIE. (2019). *Taking a multisectoral, one health approach: A tripartite guide to addressing zoonotic diseases in countries.* World Health Organization.
18. Woodford, N. (1998). Glycopeptide-resistant enterococci: A decade of experience. *Journal of Medical Microbiology, 47*(10), 849–862. https://doi.org/10.1099/00222615-47-10-849
19. Zommiti, M., Ferchichi, M., Manai, M. (2020). Probiotic characteristics of *Lactobacillus* strains isolated from fermented Tunisian food and assessment of their ability to withstand food processing conditions. *Frontiers in Microbiology, 11*, 218. https://doi.org/10.3389/fmicb.2020.00218