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Research Article

Antibiotic Resistance Profile of Indigenous *Streptococcus thermophilus* and *Lactobacillus bulgaricus* Strains Isolated from Traditional Yogurt

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Antibiotic resistance signifies a safety hazard to public health. Lactic acid bacteria, particularly, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, are useful organisms responsible for dairy fermentation. In keeping with this, they may transfer antibiotic resistance to human population. We conducted a study exanimated the antibiotic resistance pattern and distribution of antibiotic resistance genes of the *S. thermophilus* and *L. bulgaricus* strains isolated from traditional yogurt samples. Fifty-five traditional yogurt samples were collected, and *S. thermophilus* and *L. bulgaricus* strains were isolated using the microbial culture. The disk diffusion method in agar wells was utilized to assess the antibiotic resistance pattern of isolates against 13 antibiotic agents. The distribution of antibiotic resistance genes was assessed using conventional PCR. *Lactobacillus bulgaricus* and *S. thermophilus* were identified in 94.54% (52/55) and 87.27% (48/55) of yogurt samples, respectively. Both *L. bulgaricus* and *S. thermophilus* bacteria were simultaneously identified in 72.72% of samples. *Streptococcus thermophilus* isolates harbored the highest antibiotic resistance rate against tetracycline (31.25%), ampicillin (31.25%), gentamicin (25%), erythromycin (25%), penicillin (12%), and ciprofloxacin (20.83%). *Lactobacillus bulgaricus* isolates harbored the highest resistance rate against tetracycline (9.61%), ampicillin (9.61%), gentamicin (9.61%), and erythromycin (7.69%). *Streptococcus thermophilus* (33.33%) isolates harbored the higher distribution of multidrug resistance than *L. bulgaricus* (11.53%). *aacA-D* (20.83%), *tetK* (16.66%), *ermA* (14.58%), *blaZ* (14.58%), and *gyrA* (12.50%) were the most commonly detected antibiotic resistance genes in *S. thermophilus*. *AacA-D* (3.84%) and *tet K* (3.84%) were the most commonly detected antibiotic resistance genes in *L. bulgaricus*. *Lactobacillus bulgaricus* isolates did not harbor *tetM*, *cat1*, *rpoB*, *vanA*, *linA*, and *strA-strB* antibiotic resistance genes. *Streptococcus thermophilus* and *L. bulgaricus* strains used in traditional yogurt production can harbor antibiotic resistance genes and subsequently disseminate the resistance to human beings. Monitoring antibiotic resistance in fermented foods should be a common inspection for food quality.

1. Introduction

Nowadays, antibiotic prescription in livestock husbandry has emerged because of extensive usage of antimicrobial agents to ensure disease treatment and control and also as a growth. As a result, the majority of bacterial strains will show high rates of resistance against commonly prescribed antimicrobial agents [1–3].

Lactic acid bacteria (LAB) are presently classified in the phylum Firmicutes, class Bacilli, and order Latobacillales. LAB genera comprise Lactococcus, Lactobacillus, Pediococcus, Leuconostoc, Aerococcus, Streptococcus, Carnobacterium, Alloiococcus, Enterococcus, Dolosigranulum, Tetragenococcus, Oenococcus, Weissella, and Vagococcus [4, 5], Among them, *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus bulgaricus* (*L. bulgaricus*)

are frequently used in yogurt lactic acid fermentation [5]. Fermentation procedures caused by *S. thermophilus* and *L. bulgaricus* mainly yield by-products with different health-promoting effects, such as protection against infectious agents, antimicrobial, immunomodulatory, antioxidant, antiobesity, antianxiety, antiallergenic, and antidiarrheal effects [6], they also enhanced the bioavailability of vitamins and minerals [6]. As a result, the consumption of this category of food products has received more attention. Traditional yogurt samples are mainly produced in home condition, without any device- and factory-based procedure [7, 8]. Iranian traditional yogurt is made from raw sheep or cow milk [9] after adequate starter fermentation. Traditionally, yogurt production starts with milk boiling, microbial starter addition, and subsequent incubation period at 40–45°C [10]. Traditional yogurt with firm consistency and cooked flavor will produce after 12 h. This kind of traditional yogurt is a source of different LAB [10].

Some studies showed the presence of antibiotic resistance amongst the LAB bacteria [11]. In addition, in some cases, several antibiotic resistance genes have been detected in LAB bacteria [5]. Thus, though LAB has a long history of being extensively used in fermented food production and was commonly documented as safe, some of them presented high rates of antibiotic resistance [12]. Recently, numerous researchers have assessed the role of LAB as reservoirs of antibiotic resistance similar to those found among human pathogens [13]. The main issue related to LAB is that they could transfer antibiotic resistance to foodborne and pathogenic bacteria [14].

Foods with animal origins are considered as one of the major routes of transmission of antibiotic-resistant bacteria to human populations [15–17]. In this regard, fermented dairy products that are not heat treated before consumption (such as yogurt), may be considered as a vehicle for antibiotic-resistant bacteria [18]. Although the antibiotic resistance rate reported for LAB isolated from food is low, but it covers a wide range of antibiotic agents, including tetracyclines, aminoglycosides, macrolides, penicillin, quinolones, cephalosporins, and β-lactams [19]. Most antibiotic-resistant LAB harbored several genes that encode resistance against diverse antibiotic classes, such as tetracycline (*tetK* and *tetM*), aminoglycosides (*aadA-D*), erythromycin (*ermA*), penicillin (*blaZ*), chloramphenicol (*cat1*), macrolides (*gyrA*), rifampicin (*rpoB*), vancomycin (*vanA*), lincomycins (*linA*), nitrofurantoin (*nfsA*), and streptomycin (*strA-strB*) [17, 18]. These types can easily transmit antibiotic resistance encoding genes to foodborne and pathogenic bacteria. Thus, it is essential to assess the antibiotic resistance properties of LAB isolated from fermented dairy products.

Rare data are available on the prevalence and antimicrobial resistance of LAB bacteria isolated from fermented dairy samples. Thus, the present survey was carried out to determine the prevalence, antibiotic resistance pattern, and distribution of antibiotic resistance genes of the *L. bulgaricus* and *S. thermophilus* bacteria isolated from traditional yogurt samples.

2. Materials and Methods

2.1. Samples. A total of 55 different types of traditional yogurt samples (10 ml each) [1] were randomly collected from Zanjan province, Iran. All samples had normal physical characteristics of the traditional yogurt and did not have any unusual odor, color, or taste. Samples were taken using sterile tubes and transferred to laboratory. Samples were transferred to the laboratory using a small refrigerator at 4°C.

2.2. Strain Isolation and Identification. Ten grams of collected traditional yogurt samples were homogenized with 90 ml of (0.1% v/v) peptone water (Merck, Darmstad, Germany) and homogenized using a Stomacher 400 W (Interscience, Saint-Nom, France) for 2 min [18]. After that, 0.1 ml of the dilution was spread on de Man, Rogosa, and Sharpe (MRS) Agar (Merck, Darmstad, Germany) and M17 Agar (Merck, Darmstad, Germany). The M 17 agar plates were incubated aerobically for 48 h at 37°C, and the MRS agar plates were incubated anaerobically for 72 h at 42°C. An Aerogen agent (Oxoid, Basingstoke, UK) was used to produce the anaerobic condition. The morphologically suitable growing colonies were transferred to Elliker Broth (Difco, Fluka, France) and incubated at 42°C (for *L. bulgaricus*) and 37°C (for *S. thermophilus*). Finally, several biochemical tests, including Gram staining, catalase, oxidase, indole production, and sugars fermentation were applied for bacterial identification [19]. Motility of isolates was also determined [19].

DNA was extracted from colonies. Briefly, *S. thermophilus* and *L. bulgaricus* isolates were subcultured on TSB. Then, DNA extraction was performed according to the criteria presented in kit (Thermo Fisher, Germany). After DNA extraction, its purity and quality were checked by standard assays [20, 21]. The *16S rRNA* gene was used to identify bacterial isolates using universal primers 27F (5′-AGA GTT TGA TCM TGG CTC AG3′) (Pishgaman Enteghal Gene, Iran) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′). The acquired *16S rRNA* sequences of the isolates were aligned with NCBI GenBank database using the BLAST algorithm (https://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi), so the*16S rRNA* sequences of the isolates were registered in NCBI.

2.3. Antibiotic Resistance Analysis. Streptococcus thermophilus and *L. bulgaricus* antibiotic resistance was checked according to the simple disk diffusion method (Kirby Baeur) [22, 23]. Briefly, bacteria were cultured in Tryptone Soya broth (TSB, Merck, Germany) at 37°C (for *S. thermophilus*) and 42°C (for *L. bulgaricus*) for 48 h. Determined bacteria with signified concentrations (10^6 CFU/mL) were cultured on Mueller-Hinton agar (Merck, Germany) [22]. Different antibiotic discs, including chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), erythromycin (15 μg), tetracycline (30 μg), nitrofurantoin (300 μg), ampicillin (10 μg), rifampin (5 μg), vancomycin (30 μg) [19],

penicillin(10 μg), lincomycin (15 μg), kanamycin (30 μg), and streptomycin (10 μg) [22] (Oxoid, UK) were placed on the media contained bacteria. Formerly, all media were incubated at 42°C (for *L. bulgaricus*) and 37°C (for *S. thermophilus*) for 24–48 h. The diameter of the growth inhibition zone surround each disk was measured and compared with the standard guidelines [24]. *Streptococcus thermophilus* (ATCC 19258) and *Lactobacillus bulgaricus* (ATCC 11842) were used as control organisms in this test.

2.4. Determination of Antibiotic Resistance Genes. DNA was extracted from colonies. Briefly, *S. thermophilus* and *L. bulgaricus* isolates were subcultured on TSB. Then, DNA extraction was performed according to the criteria presented in kit (Thermo Fisher, Germany). After DNA extraction, its purity and quality were checked by standard assays [20, 21]. Antibiotic resistance genes were detected using the polymerase chain reaction (PCR) (Table 1) [25–30]. Eppendorf DNA thermocycler (Hamburg, Germany) was applied in this regard. Totally, 15 μl of PCR products were subjected to electrophoresis in 2.5% agarose gel contained ethidium bromide (0.1%, 0.4 μg/ml) and runned at 120 V/208 mA for about 30 to 60 min. Figure analysis was carried out by UVIDOC device (GB004, UK). *Streptococcus thermophilus* ATCC 19258 and *L. bulgaricus* ATCC 11842 were used as positive controls, and PCR-grade water (Thermo Fisher, Germany) was used as a negative control [31, 32].

PCR products of antibiotic resistance genes amongst the *S. thermophilus* and *L. bulgaricus* isolates were sequenced, and the sequences were aligned with NCBI GenBank database using BLAST algorithm (https://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) for confirmation of the results.

2.5. Data Analysis. Statistically, collected data from the experiments were examined using SPSS, 21. ver (Chicago, USA) and rendering their qualitative bases, and chi-square and Fisher's exact the two-tailed tests were applied to assess comparisons [32]. The level of significance was considered as P value<0.05 [33, 34].

3. Results

3.1. Starin Isolation and Identification. Table 2 shows the *S. thermophilus* and *L. bulgaricus* distribution amongst the examined traditional yogurt samples. *Streptococcus thermophilus* and *L. bulgaricus* were identified in 87.27% (48/55) and 94.54% (52/55) of examined traditional yogurt samples. Both *S. thermophilus* and *L. bulgaricus* bacteria were simultaneously identified in 72.72% (40/55) of examined traditional yogurt samples. Statistically significant difference was obtained between the distribution of *S. thermophilus* and *L. bulgaricus* positive and negative strains ($P < 0.05$).

All strains were identified by 16S rRNA sequences alignment with NCBI blast software and 3 of them were registered in NCBI gene bank, under the accession numbers of OP020713.1, OP002033.1, and OP002032.1. Supplementary files are available (here).

3.2. Antibiotic Resistance Pattern of S. thermophilus and L. bulgaricus Isolates. Table 3 shows the antibiotic resistance pattern of *S. thermophilus* and *L. bulgaricus* isolates. *Streptococcus thermophilus* isolates harbored the highest prevalence of resistance against tetracycline (31.25%), ampicillin (31.25%), gentamicin (25%), erythromycin (25%), penicillin (12%), and ciprofloxacin (20.83%). They harbored the lowest prevalence of resistance against vancomycin (2.08%) and lincomycin (4.16%). *Lactobacillus bulgaricus* isolates harbored the highest prevalence of resistance against tetracycline (9.61%), ampicillin (9.61%), gentamicin (9.61%), and erythromycin (7.69%), and they harbored the lowest prevalence of resistance against penicillin (5.17%), ciprofloxacin (5.17%), and nitrofurantoin (5.17%). *Lactobacillus bulgaricus* isolates did not show any resistance toward chloramphenicol, rifampin, vancomycin, lincomycin, kanamycin, and streptomycin antibiotic agents. Images for disk diffusion results are available in supplementary files.

3.3. Distribution of Antibiotic Resistance Genes. Table 4 shows the distribution of antibiotic resistance genes among the *S. thermophilus* and *L. bulgaricus* isolates. Amongst the *S. thermophilus* isolates, *aadA-D* (20.83%), *tetK* (16.66%), *ermA* (14.58%), *blaZ* (14.58%), and *gyrA* (12.50%) were the most commonly detected antibiotic resistance genes. *Streptococcus thermophilus* isolates did not harbor *vanA* antibiotic resistance gene. Amongst the *L. bulgaricus* isolates, *aacA-D* (3.84%) and *tetK* (3.84%) were the most commonly detected antibiotic resistance genes. *Lactobacillus bulgaricus* isolates did not harbor *tetM*, *cat1*, *rpoB*, *vanA*, *linA*, and *strA-strB* antibiotic resistance genes.

The results of alignment of sequences of PCR products of antibiotic resistance genes (*aadB*, cat1, *ermA*, *gyrA, nfsA, tetM*, rpoB, blaZ, and *tetK*) confirmed the results. Supplementary files are available (here).

4. Discussion

Antibiotic resistance dissemination by food is a thoughtful public health topic, and antibiotic resistance in fermented dairy products is a component of this paradigm. *Streptococcus thermophilus* and *L. bulgaricus* generally recognized as safe (GRAS) are the main starter cultures in traditional yogurt production [37]. However, their probiotic properties have introduced them as main food additives, and previous reports established a possible LAB pathogenic relevance as carriers of antibiotic resistance [38–42].

The present study showed that the *S. thermophilus* and *L. bulgaricus* bacteria were identified in 87.27% and 94.54% of examined traditional yogurt samples of Zanjan province, Iran. Both *S. thermophilus* and *L. bulgaricus* were also available in traditional yogurt samples produced in Sri Lanka [43], Poland [40], Serbia [44], and Iran [45]. *Lactobacillus bulgaricus* was more prevalent among the examined traditional yogurt samples. This part of our study was supported by the reports conducted by Alia et al. [46] and Xu et al. [47]. This may be related to the higher survival potential of *L. bulgaricus* than *S. thermophilus* in traditional yogurt samples [35, 46–48].

Table 1: PCR conditions for detection of antibiotic resistance genes amongst the *S. thermophilus* and *L. bulgaricus* isolates [25–30].

Target gene	Sequences (5′-3′)	Size (bp)	Thermal cycles	Volume (50 μL)
AadA-D	F: TAA-TCC-AAG-AGC-AAT-AAG-GGC R: GCC-ACA-CTA-TCA-TAA-CCA-CTA	227	1 cycle: 5 min at 94°C	PCR buffer (10X): 5 μL $Mgcl_2$: 1.5 mM
ermA	F: AAG-CGG-TAA-ACC-CCT-CTG-A R: TTC-GCA-AAT-CCC-TTC-TCA-AC	190	25 cycles: 60 s at 94°C 70 s at 55°C 60 s at 72°C	dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U
tetK	F: GTA-GCG-ACA-ATA-GGT-AAT-AGT R: GTA-GTG-ACA-ATA-AAC-CTC-CTA	360	1 cycle: 10 min at 72°C	DNA: 2.5 μL
tetM	F: AGT-GGA-GCG-ATT-ACA-GAA R: CAT-ATG-TCC-TGG-CGT-GTC-TA	158	1 cycle: 6 min at 94°C 34 cycles: 50 s at 95°C 70 s at 55°C 60 s at 72°C 1 cycle: 8 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_{2:}$ 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL
blaZ	F: ACT-TCA-ACA-CCT-GCT-GCT-TTC R: TGA-CCA-CTT-TTA-TCA-CAA-CC	490	1 cycle: 5 min at 94°C 30 cycles: 20 s at 94°C 30 s at 60°C 90 s at 72°C 1 cycle: 5 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_{2:}$ 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL
cat1	F: AGT-TGC-TCA-ATG-TAC-CTA-TAA-CC R: TTG-TAA-TTC-ATT-AAG-CAT-TCT-GCC	547	1 cycle: 8 min at 94°C 32 cycles: 60 s at 95°C 70 s at 55°C 120 s at 72°C 1 cycle: 8 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_{2:}$ 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL
gyrA	F: AAT-GAA-CAA-GGT-ATG-ACA-CC R: TAC-GCG-CTT-CAG-TAT-AAC-GC	223	1 cycle: 10 min at 94°C 25 cycles: 20 s at 94°C 20 s at 52°C 50 s at 72°C 1 cycle: 5 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_{2:}$ 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL
rpoB	F: ACC-GTC-GTT-TAC-GTT-CTG-TA R: TCA-GTG-ATA-GCA-TGT-GTA-TC	460	1 cycle: 5 min at 94°C 32 cycles: 60 s at 94°C 45 s at 56°C 60 s at 72°C 1 cycle: 10 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_{2:}$ 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL
vanA	F: GGG-AAA-ACG-ACA-ATT-GC R: GTA-CAA-TGC-GGC-GTT-A	732	1 cycle: 5 min at 94°C 32 cycles: 60 s at 94°C 60 s at 50°C 60 s at 72°C 1 cycle: 10 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_{2:}$ 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL

Table 1: Continued.

Target gene	Sequences (5′-3′)	Size (bp)	Thermal cycles	Volume (50 μL)
linA	F: GGT-GG-CTG-GGG-GGT-AGA-TGT-ATT-AAC-TGG R: GCT-TCT-TTT-GAA-ATA-CAT-GGT-ATT-TTT-CGA	323	1 cycle: 6 min at 94°C 30 cycles: 60 s at 95°C 60 s at 57°C 60 s at 72°C 1 cycle: 10 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_2$: 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL
nfsA	F: ATT-TTC-TCG-GCC-AGA-AGT-GC R: AGA-ATT-TCA-ACC-AGG-TGA-CC	1036	1 cycle: 2 min at 94°C 35 cycles: 30 s at 95°C 30 s at 55°C 60 s at 72°C 1 cycle: 5 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_2$: 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL
strA-strB	F: TGA-ATC-GCA-TTC-TGA-CTG-GTT R: GCT-AGA-TCG-CGT-TGC-TCC-TCT	1571	1 cycle: 2 min at 94°C 30 cycles: 60 s at 95°C 60 s at 58°C 60 s at 72°C 1 cycle: 5 min at 72°C	PCR buffer (10X): 5 μL $Mgcl_2$: 1.5 mM dNTP: 200 μM Primer F: 0.5 μM Primer R: 0.5 μM Taq DNA polymerase: 1.25 U DNA: 2.5 μL

Table 2: *S. thermophilus* and *L. bulgaricus* distribution amongst the examined traditional yogurt samples.

Type of samples	*N.* collected	*N.* positive for LAB bacteria (%)		
		S. thermophilus	*L. bulgaricus*	*S. thermophilus* + *L. bulgaricus*
Traditional yogurt	55	48 (87.27)	52 (94.54)	40 (72.72)
Status	Strains distribution (%)		χ^2	*P*-value
	S. thermophilus	*L. bulgaricus*		
Present	48 (87.27)	52 (94.54)	3.96	0.047
Absent	7 (12.72)	3 (5.45)		

Examined bacteria harbored a high prevalence of resistance against tetracycline, ampicillin, gentamicin, erythromycin, penicillin, and ciprofloxacin. Up to now, only a few investigations have focused on the identification of antibiotic resistance in *L. bulgaricus* than *S. thermophilus* strains isolated from traditional yogurt. *Lactobacillus bulgaricus* strains isolated from traditional yogurt samples in the present study harbored a lower rate of antibiotic resistance than *S. thermophilus* isolates. This part of our survey was supported by the researchers conducted in Turkey [49], Germany [50], and China [51]. Zhou et al. [36] showed that the prevalence of resistance of *L. bulgaricus* strains isolated from Chinese yogurt samples against penicillin G, roxithromycin, ampicillin, chlortetracycline, tetracycline, chloramphenicol, lincomycin, streptomycin, neomycin, and gentamycin was 23.50%, 64.70%, 29.40%, 47.10%, 88.20%, 88.20%, 94.10%, 94.10%, 94.10%, and 94.10%, respectively. The prevalence of resistance of *S. thermophilus* strains against similar antibiotic agents was 0%, 0%, 44.40%, 27.80%, 44.40%, 88.90%, 88.90%, 66.70%, and 88.90%, respectively. A review study conducted by Zarzecka et al. [52] showed that the majority of *L. bulgaricus* and *S. thermophilus* strains isolated from dairy products harbored considerable resistance against ampicillin, tetracycline, gentamicin, erythromycin, lincomycin, and streptomycin antibiotic agents. Tavşanlı et al. [53] reported a higher antibiotic resistance rate of *S. thermophilus* than *L. bulgaricus* strains. They showed that *L. bulgaricus* strains harbored complete susceptibility against quinupristin/dalfopristin, ampicillin, vancomycin, linezolid, gentamicin, streptomycin, tetracycline, cefotaxime, kanamycin, and penicillin. However, they showed that *S. thermophilus* strains harbored a high prevalence of resistance toward quinupristin/dalfopristin (11.50%), ampicillin (61.50%), vancomycin (3.80%), linezolid (30.70%), gentamicin (65.30%), ciprofloxacin (46.10%), streptomycin (69.20%), tetracycline (34.60%), clindamycin (61.50%), erythromycin (50%), cefotaxime (34.10%), kanamycin (53.80%), chloramphenicol (23%), and penicillin (23%), which was following our findings. Different studies reported dissimilar LAB resistance rates. This inconsistency may be due to differences in the types and origin of the isolates, and antibiotic resistance identification

Table 3: Antibiotic resistance pattern of *S. thermophilus* and *L. bulgaricus* isolates.

Sample type/bacteria (*N.* isolated bacteria)		*N.* isolates harbored resistance against each antibiotic agent (%)												
		C30*	Cip	G10	E15	T30	Nit	A10	Rif	Van	P10	Lin	Kan	S10
Traditional yogurt	*S. thermophilus* [35]	3 (6.25)	10 (20.83)	12 (25)	12 (25)	15 (31.25)	8 (16.66)	15 (31.25)	5 (10.41)	1 (2.08)	12 (25)	2 (4.16)	4 (8.33)	5 (10.41)
	L. bulgaricus [36]	—	3 (5.17)	5 (9.61)	4 (7.69)	5 (9.61)	3 (5.17)	5 (9.61)	—	—	3 (5.17)	—	—	—

*C30: chloramphenicol (30 μg), Cip: ciprofloxacin (5 μg), G10: gentamicin (10 μg), E15: erythromycin (15 μg), T30: tetracycline (30 μg), Nit: nitrofurantoin (300 μg), A10: ampicillin (10 μg), Rif: rifampin (5 μg), Van: vancomycin (30 μg), P10: penicillin (10 μg), Lin:lincomycin (15 μg), Kan: kanamycin (30 μg), and S10: streptomycin (10 μg).

Table 4: Antibiotic resistance gene pattern of *S. thermophilus* and *L. bulgaricus* isolates.

Sample type/bacteria (*N.* isolated bacteria)		*N.* isolates harbored resistance against each antibiotic agent (%)											
		*aadA-D**	*ermA*	*tetK*	*tetM*	*blaZ*	*cat1*	*gyrA*	*rpoB*	*vanA*	*linA*	*nfsA*	*strA-strB*
Traditional yogurt	*S. thermophilus* [35]	10 (20.83)	7 (14.58)	8 (16.66)	2 (4.16)	7 (14.58)	1 (2.08)	6 (12.50)	1 (2.08)	—	1 (2.08)	3 (6.25)	2 (4.16)
	L. bulgaricus [36]	2 (3.84)	1 (1.92)	2 (3.84)	—	1 (1.92)	—	1 (1.92)	—	—	—	1 (1.92)	—

**aacA-D*: aminoglycosides, *ermA*: erythromycin, *tetK* and *tetM*: tetracyclines, *blaZ*: penicillins, *cat1*: chloramphenicol, *gyrA*: macrolides, *rpoB*: rifampin, *VanA*: vancomycin, *LinA*: lincomycin, *nfsA*: *L.* nitrofurantoin, and *strA-strB*: streptomycin.

methods, availability or nonavailability of antibiotics, the level of strict rules in prescribing antibiotics, and the opinion of physicians and veterinarians on prescribing antibiotics. The primary origin of antibiotic resistance in the strains isolated in this study is not known. Nevertheless, the possibility of transferring antibiotic resistance from livestock and also the stuff of traditional yogurt production centers is expected. Some of the antibiotics used in this study have a hospital origin and are never used in the treatment of livestock diseases in Iran (ciprofloxacin, rifampin, vancomycin, and kanamycin) [21, 27]. The reason for the prevalence of antibiotic resistance against these antibiotics was probably the transfer of resistance from human pathogenic strains to LAB during the milking and processing of milk into traditional yogurt [33]. Chloramphenicol resistance was only identified in 6.25% of *S. thermophilus* strains. Chloramphenicol is an illicit drug with a limited prescription. The use of this antibiotic illegally is carried out only in poultry farms in Iran. Probably the reason for the low prevalence of antibiotic resistance against chloramphenicol is the keeping of cattle, sheep, and goats traditionally next to poultry and the transfer of chloramphenicol-resistant strains from poultry to milk-producer animals [52–55].

Antibiotic resistance is an undesirable trait among LAB since they are used as starters in the fermentation process and also some being used as probiotics. Findings of the present survey showed that *aacA-D*, *tetK*, *ermA*, *blaZ*, and *gyrA* were the most commonly detected antibiotic resistance genes among the *S. thermophilus,* and *aacA-D* and *tetK* harbored the higher distribution in *L. bulgaricus* strains. Lower distribution of antibiotic resistance genes was reported amongst the *L. bulgaricus* strains. The literature searches showed that *tet*, *blaZ*, *erm*, and *aac* were more frequently detected in the LAB isolated from dairy products [41, 56–59]. A survey conducted by Zhou et al. [36] indicated that the *tetM* was detected in 6.67% of tetracycline-resistant *L. bulgaricus* and 25% of *S. thermophilus* strains. Other antibiotic resistance genes (*tetK*, *str*, *cat*, and *aac-6-aph-2*) were detected in their survey. Flórez and Mayo [41] stated that *tetS* and *ermB* antibiotic resistance genes were detected in all LAB bacteria. Resistance to aminoglycosides is mainly facilitated by the presence of *aacA-D* gene. Membrane impermeability has been considered the main LAB aminoglycosides resistance mechanism because most of the species lack the cytochrome-mediated electron transport that can mediate drug uptake [39]. Other nonspecific mechanisms, including multidrug transporters and defective cell wall autolytic systems, may contribute to LAB antibiotic resistance [57, 60, 61]. Other studies described that the *aph*, *aph(3)*, *ant(6)*, and *aac(6)–aph(2)* presence may induce aminoglycosides resistance [13, 62, 63]. *aadA-D* gene was detected in 20.83% and 3.84% of *S. thermophilus* and *L. bulgaricus* bacteria of the present study, respectively. Prevalence of resistance against gentamicin and kanamycin was found in 25% and 8.38% of *S. thermophilus* and 9.61% and 0% of *L. bulgaricus* strains, respectively. The effect of low pH of the MRS agar (6.2 ± 0.2) might cause some decrease in the aminoglycosides antimicrobial effect (optimum pH, 7.8) [49]. Similarly, Li et al. [51] reported that *sul*, *tetM*, *str*, and *aac* genes were present in *S. thermophilus* and *L. bulgaricus* strains isolated from fermented milk products. Our findings showed that isolated bacteria harbored a higher antibiotic resistance pattern than the distribution of antibiotic resistance encoding genes. This may be due to the fact that the same gene may be responsible for resistance to several families of antibiotics in the same strain. Thus, it is logical that the distribution of antibiotic resistance genes was lower in antibiotic-resistant bacteria. The probable reason for the occurrence of antibiotic resistance in LAB bacteria is maybe low level of hygiene maintained during the processing and storage of traditional yogurt samples, inoculation procedure, raw material quality, and the utensil hygiene. Higher prevalence of *L. bulgaricus* may be due to the higher resistance of this organism against acidic pH. As, traditional yogurt samples have acidic pH, only acid-resistance organisms can survive and growth on them. *L. bulgaricus* strains are more resistant than *S. thermophilus* [64].

5. Conclusion

In conclusion, *S. thermophilus* and *L. bulgaricus* strains were detected in 87.27% and 94.54% of traditional yogurt samples. The considerable prevalence of *S. thermophilus* and *L. bulgaricus* strains was accompanied by the high rate of bacterial resistance toward commonly used antibiotic agents, particularly, tetracycline, ampicillin, gentamicin, erythromycin, penicillin, and ciprofloxacin. The findings may show the high antibiotic resistance of *S. thermophilus* and *L. bulgaricus* and the potential role of traditional yogurt samples in the transmission of antibiotic resistance to the human population. Some strains harbored different antibiotic resistance genes, particularly *aacA-D*, *tetK*, *ermA*, *blaZ*, and *gyrA*. Some strains also harbored multidrug resistance. The higher antibiotic resistance of *S. thermophilus* strains highlighted the need for strict monitoring and regulation in the food industry. Additional studies should focus on testing the transferability of genetic determinants between LAB bacteria and the human population. Assessment of the LAB consumption safety must be guided by establishing criteria and regulations, and standardized methods for premarket biosafety testing and postmarket surveillance.

Data Availability

The data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Bahar Moghimi and Maryam Ghobadi Dana involved in the designing, conceptualization, supported the research, and performed the culture and molecular analysis and statistical analysis. Abbasali Motalebi supported the study and carried out the disk diffusion. Amirali Anvar carried out the bacterial isolation and identification. The authors have read and

approved the final manuscript. Reza Shapouri and Maryam Jalili carried out the sample collection, DNA extraction, and disk diffusion. The authors have read and confirmed the final manuscript.

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Supplementary Materials

Images for disk diffusion results and *L. bulgaricus* colonies are available in supplementary files. The sequences of PCR products of *aadB*, cat1, *ermA*, *gyrA*, *nfsA*, *rpoB*, *tetM*, blaZ, and *tetK* and the results of alignment of sequences of PCR products of *aadB*, cat1, *ermA*, *gyrA*, *nfsA*, *tetM*, and *tetK* were placed in supplementary files. All strains were identified by 16S rRNA sequences alignment with NCBI blast software and 3 of them were registered in NCBI gene bank, under the accession numbers of OP020713.1, OP002033.1, and OP002032.1. Supplementary files are available. (*Supplementary Materials*)

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