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RESEARCH ARTICLE

Antibiotic Resistance Profile of Yogurt Bacteria Exposed to Various Stress Conditions

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ABSTRACT

This study aims to reveal the differences that may occur in the susceptibilities of 2 yogurt bacteria strains, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, to antibiotics under 2 different durations and 3 different stress conditions. The study also introduces new approaches to reduce potential issues in the fermented milk industry containing antibiotics in milk. To this end, the bacteria were exposed to 2 different low-pressure, low-temperature, and magnetic field activities under 2 different durations. The research concluded that as the severity of the applied stress conditions and application period increase, the antibiotic susceptibility of the bacteria decreases, and resistance to certain antibiotics develops ($p<0,05$). In the conclusion of the 3 different stress applications, it was found that the bacteria had the highest resistance to antibiotics in the magnetic field applications. In these 3 different stress applications, *S. thermophilus* showed the highest resistance to lincomycin, cephalexin, and streptomycin; *L. delbrueckii* subsp. *bulgaricus* developed resistance to streptomycin, erythromycin, and chloramphenicol. Of the 2 yogurt bacteria, L. delbrueckii subsp. bulgaricus developed a resistance to more antibiotics than *S. thermophilus* after the stress applications; the developed resistance was also more substantial than that of *S. thermophilus*.

Keywords: Magnetic Field, Streptomycin, Stress, Yogurt Bacteria

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Çeşitli Stres Koşullarına Maruz Kalan Yoğurt Bakterilerinin Antibiyotik Direnç Profili

ÖZ

Bu araştırmada iki farklı süre ve üç farklı stres koşulları altında yoğurt bakterileri *S. thermophilus* ve L. delbrueckii subsp. *bulgaricus* suşlarının, antibiyotiklere karşı olan duyarlılıklarında meydana gelebilecek değişimlerin belirlenmesi ve fermente süt endüstrisinde antibiyotikli süt kullanımına bağlı ortaya çıkan sorunların azaltılmasında yeni yaklaşımların ortaya konulması amaçlanmıştır. Bu amaçla bakteriler iki farklı sürede olacak şekilde, iki farklı düşük basınç, düşük sıcaklık ve manyetik alan uygulamalarına tabi tutulmuşlardır. Araştırma sonucunda uygulanan stres koşullarının şiddeti ve uygulama zamanı arttıkça bakterilerde oluşan antibiyotik duyarlılığının azaldığı, bazı antibiyotik türlerine karşı ise direnç gelişiminin ortaya çıktığı tespit edilmiştir ($p<0,05$). Üç farklı stres uygulaması sonucunda bakterilerin antibiyotik türlerine karşı en fazla direnci manyetik alan uygulamalarında oluşturduğu tespit edilmiştir. Üç farklı stres uygulamasında *S. thermophilus* en fazla linkomisin, sefaleksin ve sitreptomisine, *L. delbrueckii* subsp. *bulgaricus* ise, Streptomisin, Erythromycin ve Chloramphenicole karşı direnç geliştirmiştir. İki farklı yoğurt bakterisinden L. delbrueckii subsp. bulgaricus bakterisinin, *S. thermophilus*'a kıyasla stres uygulamaları sonucunda daha fazla antibiyotiğe karşı direnç geliştirdiği, ayrıca gelişen direncin de *S. thermophilus*'dan daha yüksek olduğu araştırma sonucunda tespit edilmiştir.

Anahtar Kelimeler: Manyetik Alan, Streptomisin, Stres, Yoğurt Bakterileri

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INTRODUCTION

One of the most significant issues with fermented milk production recently has been the use of antibiotics in milk during the dairy process and the resulting loss in quality (Brady 1988). Specifically, a portion of the antibiotics (30–80%) used in the treatment of mastitis in dairy animals can pass into milk (Chiders and Jones 1985). Even if the antibiotic concentration passing into milk is insignificant, it slows down—and can even stop—starter culture/acid production in dairy products. This can cause problems in producing various dairy products, such as yogurt, ayran, and cheese and cause serious quality loss (Jones and Seymour 1988).

Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus* are among the most used starter bacteria in the production of fermented dairy products (primarily yogurt and ayran) (Song and Aryana 2014). In the fermentation of milk, lactic acid bacteria (LAB) produce many products, such as lactic acid, other organic acids, bacteriocins, exopolysaccharides, and vitamins, and they are the foundation of the production and quality characteristics of fermented milk products (Devanthi et al. 2018). LAB can undergo various abiotic and biotic changes (e.g., acidic, thermal, osmotic, oxidative, and other stresses) that seriously affect metabolic activity and production efficiency (Papadimitriou et al. 2016).

As in other activities, LAB identify all of the surrounding inconvenient, physical, chemical, and biological conditions as stress. These stress factors can affect the cell wall and membrane of LAB (Lakhotia 2001). In response to these various stress conditions, LAB use multi-faceted strategies to resist the damage incurred by these challenging environments (Shin et al. 2018; Wei et al. 2019; Yang et al. 2023). LAB resist these stress environments using a system called cross-protection metabolism. The stress response developed against these stress factors enables LAB survival, and these factors can also cause changes in the bacteria's biological activities (acid formation, antibiotic resistance, etc.) (Chen et al. 2017; Shin et al. 2018; Kulkarni et al. 2018; Zhang et al. 2018).

Milk containing antibiotics used in the production of fermented dairy products leads to many substantial issues— from loss of quality in the end product to an inability to obtain a product in the first place. Previous studies have shown LAB's behaviors, metabolism, and the changes in the metabolites they produce under different stress conditions. This study aims to identify the changes that might occur in the susceptibilities of 2 yogurt bacteria to antibiotics under similar stress conditions and introduce new approaches to decrease issues related to using milk-containing antibiotics in the fermented milk industry.

MATERIALS and METHOD

Starter Cultures

S. thermophilus (DSM 20617, ATCC19258) and *L. delbrueckii* subsp. *bulgaricus* (DSM 20081, ATCC 11842) strains were used in this study.

The two different starter culture types were first incubated inside MRS broth (110661, Merck Millipore, Germany) at 37°C under anaerobic conditions for 48–72 hours. At the end of the incubation period, the bacteria developed inside the broth were inoculated into *Streptococcus* agar (11007, Merck Millipore, Germany) and *Lactobacillus bulgaricus* agar (17154, Merck Millipoe, Germany) growth mediums and incubated again at 37°C under anaerobic conditions for 48–72 hours.

Stress Applications

Low-pressure application

The incubated cultures were then subjected to a low-pressure application for 1 to 2 hours under 3 different pressures inside a cabin designed by Biosan (Konya, Türkiye). The environmental conditions in the application were as follows: temperature: 30°C; moisture: 55.7%; oxygen concentration: 0.06%; and carbon dioxide: 0.13 ppm.

Low-temperature application

The cultures were also subjected to a low-temperature application at 3 different temperatures and 2 different durations after the incubation process; a 0°C temperature application was carried out inside a refrigerator (Beko, D948ANEK, Türkiye), a –18°C temperature application was performed inside a deep freezer (Uğur ED 560 DS, Türkiye), and a –75 °C temperature application was implemented inside an ultra-freezer (Thermo Scientific Forma 900 Series, USA). The environmental conditions during the applications were as follows: temperature: 0°C; moisture: 65.1%; oxygen concentration: 14.3%; carbon dioxide: 0.733 ppm; temperature: –18 °C; moisture: 68.6%; oxygen concentration: 9.65%; carbon dioxide: 0.466 ppm; temperature: –75°C; moisture: 48.6%; oxygen concentration: 2.23%; and carbon dioxide: 0.33 ppm.

Magnetic field application

The study was conducted using a mechanism designed specifically for this purpose, which was made of aluminum materials (Figure 1). The rotating part of the device could move due to its rotor. However, there is a stable part inside the mechanism where the cultures are normally located. The rotating part is square prism-shaped, has 12 magnets (3 magnets on each side), and is connected to a rotator with an adjustable rotation speed. The rotation speed of the rotor was kept stable during the experiments and set to 80 rpm. The magnets were replaced after each trial; therefore, a magnetic field of 3 different strengths could be achieved. The strength of

themagnets was determined as 15, 20, and 25 μT. As a result, magnetic fields at strengths of 180, 240, and 300 μT were attained during the application. The magnetic field strength was constantly measured with the help of a Tesla meter during the research process. The environmental conditions during the applications were as follows: temperature: 30°C; moisture: 63.4%; oxygen concentration: 13.92%; and carbon dioxide: 0.652 ppm.

Antibiotic Discs

Antibiotic discs for penicillin, amoxicillin, cephalexin, erythromycin, chloramphenicol, streptomycin, lincomycin, and tetracycline (Oxoid) were used in the antibiotics susceptibility analysis.

Antibiotic Susceptibility Analysis

After the application procedures were completed, the starter bacteria in the Petri dishes were separately removed with a sterile loop and suspended in tubes containing 10 mL of sterile physiological saline (Merck, 115525, Germany) with the help of a densitometer until homogeneous turbidity was formed. The density of the obtained inoculum suspension was arranged to attain a 0.5 McFarland (8.17 Log kob/mL) standard with the help of a densitometer (Biosan, 1B, Türkiye). Subsequently, 0.1 mL of prepared inoculum was taken using a sterile pipette and inoculated into Mueller-Hinton agar (Merck, 1.05437, Germany) growth mediums. Later, the inoculum was homogenously separated on the Petri dish's surface using a sterile swap and, using sterile pliers, placed on different parts of the growth mediums at a distance, enabling the zones that would eventually grow antibiogram discs to not touch each other (Akarca et al. 2019). The Petri dishes were then placed into anaerobic jars (Merck, 113681, Germany); each jar was sealed after Anaerocult A was added to it (Merck, 113829, Germany), 3 mL of pure water was added to each section, and the samples were incubated at 42°C for 48–72 hours in a drying oven (Incucel, MMM, Germany) (Bracquart 1981). The anaerobicity of the incubation medium was checked with an Anaerotest (Merck, 132371, Germany), one piece of which was placed in each jar. The zones that formed around the disc following incubation were measured in mm in a sufficiently lighted environment using a digital caliper (Mitutoyo, IP67 0–150 mm, Japan).

Statistical Analyses

The study experiments were conducted as 2 parallel and 2 repetitions. The results were calculated using the SPSS V 27.0.0 (SPSS Inc., Chicago, IL, USA) statistical package program. The data obtained from the analyses were then evaluated using variance analysis. The significance level was determined according to Duncan's test ($p<0,05$), and the effect of the results was determined using the Pearson correlation coefficient.

RESULTS

The antibiotic susceptibility results for the *S. thermophilus* starter culture, at 2 different durations and after 3 different low-temperature applications, are given in Table 1. The interactions between antibiotic type, pressure, and interaction duration on antibiotic susceptibility were highly significant ($p<0,0001$). The interaction between antibiotic type × pressure interaction on antibiotic susceptibility was also found to be highly significant ($p<0,01$). The pressure interaction on antibiotic susceptibility also showed a very high negative correlative effect (Table 1).

As a result of the antibiotic susceptibility determined after 3 different low-pressure applications under 2 different durations on the *L. delbrueckii* subsp. *bulgaricus* starter culture, the interactions between antibiotic type, pressure, and antibiotic type × pressure on antibiotic susceptibility were found to be highly significant ($p<0,0001$). The interaction of pressure × interaction duration was also significant ($p<0,05$) on antibiotic susceptibility. In addition, the pressure interaction on antibiotic susceptibility showed a very high negative correlative effect, and antibiotic-type interaction on antibiotic susceptibility indicated a very high positive correlation (Table 2).

The antibiotic susceptibility results applied to the *S. thermophilus* starter culture at 2 different durations and 3 different temperatures are given in Table 3. It was determined that the interactions between antibiotic type, applied temperature, interaction duration, and the antibiotic type × degree of applied temperature were highly significant on antibiotic susceptibility ($p<0,0001$). Additionally, the interaction of temperature applied on antibiotic susceptibility had a high negative correlative effect (Table 3).

The interactions between antibiotic type, applied temperature, and antibiotic type × applied temperature × interaction duration on antibiotic susceptibility degree obtained after a low-temperature application to *L. delbrueckii* subsp. *bulgaricus* at 2 different durations and 3 different degrees were highly significant ($p<0,0001$). In addition, the low-temperature interaction showed a very high negative correlative effect on antibiotic susceptibility (Table 4).

The results of this study show that the interactions between antibiotic type, severity of the magnetic field, interaction duration, antibiotic type × severity of the magnetic field, and magnetic field × interaction duration on antibiotic susceptibility are highly significant ($p<0,0001$). In addition, the severity of the magnetic field interaction on antibiotic severity had a high negative correlative effect (Table 5).

The interactions between antibiotic type, magnetic field severity, and antibiotic type × applied temperature × magnetic field severity on antibiotic susceptibility degree identified after a magnetic field application to *L. delbrueckii* subsp. *bulgaricus* at 3 different durations and 3 different severities were highly significant ($p<0,0001$). Magnetic field severity

on antibiotic susceptibility also showed a high negative correlative effect (Table 6).

DISCUSSION

The susceptibility of the *S. thermophilus* starter culture to the antibiotic types used in the study differed ($p<0,05$). Before any application was initiated, it was determined that the antibiotic type that *S. thermophilus* was most susceptible to was tetracycline, with a 33.04-mm zone diameter, followed by erythromycin and penicillin, with 23.91 and 22.43-mm zone diameters, respectively. Before the applications, it was determined that the susceptibility of *S. thermophilus* to all of the antibiotic types used in the study was at an ultra-sensitive (≥18-mm zone diameter) level (Table 1).

As the severity of low pressure and application duration increased, the antibiotic susceptibilities of *S. thermophilus* decreased, but the resistance to antibiotic types increased ($p<0,05$). Especially after applying a pressure of –300 mbar for 2 hours, *S. thermophilus* showed resistance to 5 different antibiotic types, and its level of susceptibility to 3 different antibiotic types decreased from ultra-sensitive to moderately sensitive. In the conclusion of the application, after a –100 mbar pressure application for 1 hour, it was determined that the antibiotic type the bacterium was most susceptible to was tetracycline, with a zone diameter of 23.99 mm. Nonetheless, the bacteria showed the highest resistance to lincomycin, with a 9.15-mm zone diameter after a –300 mbar pressure application for 2 hours (Table 1).

The *L. delbrueckii* subsp. *bulgaricus* starter culture showed varying susceptibility to the antibiotic types used in the study ($p<0,05$). It was observed that before the applications, *L. delbrueckii* subsp. *bulgaricus* was most susceptible to the tetracycline antibiotic type (with a zone diameter of 30.14 mm) and most resistant to streptomycin (with a zone diameter of 17.59 mm). Similarly, at the beginning of the application, *L. delbrueckii* subsp. *bulgaricus* showed very high susceptibility (with a zone diameter ≥18 mm) to all antibiotic types used in the study (Table 2).

As the negative pressure and interaction duration increased, the susceptibility degree of *L. delbrueckii* subsp. *bulgaricus* to most of the antibiotic types decreased ($p<0,05$). This change mainly occurred during the –300 mbar application for 2 hours, followed by –300 mbar pressure for 1 hour and –200 mbar pressure for 2 hours. In addition, the susceptibility level (zone diameter ≥18 mm), which was very high in all antibiotics except for streptomycin at the beginning of the application, became susceptible to the 5 antibiotics used (10–14-mm zone diameter) after a –300 mbar pressure application for 2 hours. Although no difference was observed regarding the susceptibility level to tetracycline after the applications, the high susceptibility degree to streptomycin (14–16-mm zone diameter) reached the resistance (≤10-mm zone diameter) level (Table 2).

The physiological response that the different LABs exhibit to low-pressure stress varies. Most of the low-pressure stress LAB encounters impacts the cell wall (Silver 2003). However, LAB can change the nature of the cell wall and respond to this stress (Piuri et al. 2005; Koch et al. 2007); the cell wall responds by using a set of regulatory systems (Silver 2003).

The primary reaction of LAB to this stress is to produce or import tiny molecules called osmolytes (e.g., glycine betaine, choline, or proline) to balance the intracellular and extracellular difference (Molenaar et al. 1993; Glaasker et al. 1998).

The *S. thermophilus* starter culture exposed to different low temperatures showed varied susceptibility to antibiotic types used in the study ($p<0,05$). It was determined before initiating any applications that *S. thermophilus* had an ultra-sensitive (≥18-mm zone diameter) level of susceptibility to all of the antibiotic types used in the study; however, after the low-temperature applications, the susceptibility levels decreased drastically and turned into resistance in both samples (Table 3).

As the degree of low-temperature applied and application duration increased, the antibiotic susceptibilities of *S. thermophilus* decreased, but the resistance to antibiotic types increased ($p<0,05$). After the application at –75°C for 2 hours, *S. thermophilus* showed resistance to 2 different antibiotic types, and its susceptibility degree to the 5 different antibiotic types decreased from ultra-sensitive to moderately sensitive. In the conclusion of the applications, it was revealed that the antibiotic type the bacterium is most susceptible to was tetracycline, with a 28.38-mm zone diameter after the application at 0°C for 1 hour; however, the highest resistance was to cephalexin, resulting in a 9.13-mm zone diameter following an application at –65°C for 2 hours (Table 3).

L. delbrueckii subsp. *bulgaricus* exhibited different degrees of susceptibility to the antibiotic types used in the study ($p<0,05$). Before the applications began, *L. delbrueckii* subsp. *bulgaricus* showed very high susceptibility (≥18-mm zone diameter) to all antibiotic types except for streptomycin (Table 4). As the low-temperature application and interaction duration continued, the degree of susceptibility of *L. delbrueckii* subsp. *Bulgaricus* to the antibiotic types decreased ($p<0,05$). This change degree occurred the least on tetracycline; however, the most significant change was on streptomycin, followed by cephalexin and erythromycin, respectively. The bacteria developed resistance to 1 antibiotic after a –65°C low-temperature application for 1 hour and to 4 antibiotics following a –65°C low-temperature application for 2 hours (Table 4).

Exposure to low temperatures is common for LAB types in various contexts. Cold shock causes much less damage to cells compared to other stresses. LAB

produces the main reaction to cold shock with the help of cell walls, and this reaction occurs quickly in LAB by producing a subset of cell wall proteins, the heat shock proteins, or the molecular chaperons that help protein folding.

The negative effect of exposure to cold stress is primarily due to the physical impact of low temperature on cell structures and enzymatic reactions. Most LAB types react to cold shock through the temporary induction of specific protein sets called cold-induced proteins and by suppressing other proteins synthesized after exposure to stressful conditions. Such a reaction is assumed to help cells overcome the physiological stress caused by cold shock (Van de Guchte et al. 2002; Chastanet et al. 2003; Spano and Massa 2006; Fiocco et al. 2009).

The *S. thermophilus* starter culture showed different susceptibilities to the antibiotic types used in the study ($p<0,05$). Additionally, as the severity of the applied magnetic field and application duration increased, the high susceptibilities identified in the beginning decreased, and the resistance to these antibiotic types increased ($p<0,05$).

It was observed that especially after a 300-μT magnetic field application for 2 hours, the bacteria showed resistance to 6 different antibiotic types, and the susceptibility degree to 2 antibiotic types decreased from ultra-sensitive to moderately sensitive. In the different magnetic field applications, the antibiotic type the bacterium is most susceptible to was tetracycline, with a 19.99-mm zone diameter after a 180-μT magnetic field application for 1 hour. However, the bacteria showed its highest resistance to streptomycin, with an 8.01-mm zone diameter after a 300-μT magnetic field application for 2 hours (Table 5).

L. delbrueckii subsp. *bulgaricus* exhibited different degrees of susceptibility to the antibiotic types used in the study ($p<0,05$). As the magnetic field application and interaction duration continued, the susceptibility degree of the bacteria to antibiotic types decreased ($p<0,05$); this degree of change occurred the least with amoxicillin; however, the highest change was with streptomycin, followed by cephalexin and lincomycin, respectively. The bacteria developed resistance to 1 antibiotic after a 240-μT magnetic field application for 2 hours and 7 antibiotics after a 300-μT magnetic field application lasting 2 hours (Table 6).

A magnetic field causes biomolecular and chemical effects (that affect the electronic spin states of the reaction intermediates) in the cytoplasm of the lactic bacteria. This kind of effect can cause changes in intracellular ion homeostasis (Pei et al. 2006), enzyme activities (Dang et al. 2007), cell shape, cell growth (Wang et al. 2002), and cell division (Naruse 2002).

Previous research has shown that most biological tissues are diamagnetic (Liu et al. 2017). A diamagnetic response to an external magnetic effect striking it creates a magnetic induction in the opposite direction (Butler 2014). Specifically, a magnetic field of low severity and frequency affects the movement of ions along the cell membrane (Wang and Hladky 1994); the magnetic field also affects the conductance of K+ channels on cell membranes (Cecchetto et al. 2015).

In a study on the resistance of LAB to antibiotics, Aslım and Beyatlı (2004), found that 34 *S. thermophilus* strain isolates taken from yogurt samples from various villages and towns in Türkiye showed resistance to gentamicin and penicillin and that these strains were susceptible to tetracycline and chloramphenicol. Tatlı (2009), demonstrated that LAB strains isolated from traditionally produced dairy products were resistant to vancomycin, ciprofloxacin, gentamicin, erythromycin, and tetracycline. Similarly, research conducted by Kılıç (2014), found that isolates isolated from traditionally produced white cheese samples were susceptible to ampicillin, vancomycin, penicillin, gentamicin, chloramphenicol, and teicoplanin—and resistant to streptomycin and ciprofloxacin. Özteber (2013), found the highest resistance to lincomycin (25.59%) in the isolates isolated from fermented dairy products, followed by tetracycline, meropenem, ampicillin, gentamicin, erythromycin, ciprofloxacin, chloramphenicol, and vancomycin, respectively.

Table 1. Antibiotic sensitivity of *S. thermophilus* after different pressure and duration application

Type of Antibiotics	Control		Pressure											
			-100 mbar			-200 mbar				-300 mbar				
			1 Hour		2 Hour		1 Hour		2 Hour		1 Hour		2 Hour	
Penicilin	22.43±0.72^{Abc}	+++	21.24±1.17^{Aab}	+++	20.49±1.18^{Aa}	+++	15.86±0.26^{Bbc}	++	15.06±1.33^{Babc}	++	11.76±0.72^{Cb}	+	10.07±0.08^{Cb}	+
Amoxcicilin	20.66±0.80^{Abc}	+++	20.04±1.26^{ABb}	+++	19.04±0.53^{ABab}	+++	14.41±5.29^{BCbc}	++	12.32±3.93^{Cbc}	+	11.04±0.18^{Cbc}	+	9.91±0.44^{Cb}	-
Cephalexin	19.91±0.77^{Ac}	+++	18.73±1.28^{ABbc}	+++	15.70±1.18^{BCbc}	++	14.93±2.47^{Cbc}	++	12.97±1.90^{CDbc}	+	9.98±0.09^{DEc}	-	9.62±0.58^{Eb}	-
Erythromycin	23.91±2.19^{Ab}	+++	21.05±1.99^{ABab}	+++	19.17±2.05^{BCab}	+++	18.73±1.28^{BCab}	+++	16.11±0.33^{Cab}	++	11.80±0.54^{Db}	+	9.99±0.07^{Db}	-
Chloramphenicol	20.86±1.73^{Abc}	+++	18.24±0.59^{ABbc}	+++	16.53±0.99^{BCbc}	++	16.80±1.43^{BCbc}	++	14.90±1.64^{CDabc}	++	13.73±0.41^{CDa}	+	12.03±0.57^{Da}	+
Streptomycin	18.73±1.29^{Ac}	+++	14.93±2.46^{Bc}	++	13.10±2.16^{BCc}	+	11.41±1.09^{BCc}	+	10.05±0.58^{CDc}	+	10.57±0.62^{CDbc}	+	9.37±0.08^{Db}	-
Lincomycin	19.31±1.91^{Ac}	+++	17.56±1.20^{ABbc}	++	15.02±0.70^{BCc}	++	14.06±1.67^{BCbc}	++	11.49±2.92^{CDbc}	+	11.09±0.88^{CDbc}	+	9.15±0.39^{Db}	-
Tetracycline	33.04±2.34^{Aa}	+++	23.99±1.70^{Ba}	+++	20.97±2.19^{BCa}	+++	22.66±1.25^{BCa}	+++	19.31±1.26^{Ca}	+++	14.32±0.42^{Da}	++	12.47±1.48^{Da}	+
Variation					**P Value**						**r**			
Type of Antibotics(A)					<0.0001						0.111			
Pressure (P)					<0.0001						-0.784**			
Interaction Time (I)					<0.0001						-0.129			
A x P					0.01						--			
A x I					0.966						--			
P x I					0.042						--			
A x P x I					1.000						--			

a-c (→): Values shown with different letters on the same line differ from each other at the p<0.05 level, A-E (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level, ± Standard deviation,). *P* < 0.0001: Statistically too much significant, *P* =0.01: Statistically much significant, *P* =0.05: Statistically significant, **. Correlation is significant at the 0.01 level (2-tailed). ≤ 10(-): Resistant. 10- 14(+): Moderately Sensitive. 14-18(++): Sensitive. 18≥ (+++): Ultra-sensitive.

Table 2. Antibiotic sensitivity of *L. delbrueckii* subsp. *bulgaricus* after different pressure and duration application

Type of Antibiotics	Control		Pressure											
			-100 mbar			-200 mbar				-300 mbar				
			1 Hour		2 Hour		1 Hour		2 Hour		1 Hour		2 Hour	
Penicilin	23.26±0.24Abc	+++	17.05±1.35Bc	++	15.08±1.91BCcd	++	15.78±0.25BCb	++	13.13±1.01CDb	+	13.11±1.50CDcd	+	11.36±1.78Dbc	+
Amoxcicilin	21.84±0.13Ac	+++	19.36±1.85ABc	+++	17.00±1.89BCcd	++	15.82±1.02Cb	++	14.39±0.49CDb	++	11.85±2.54Dd	+	11.06±0.79Dbc	+
Cephalexin	25.93±2.06Ab	+++	23.25±1.52ABb	+++	22.17±0.63Bb	+++	18.47±0.85Cb	+++	16.52±1.37CDb	++	17.91±1.49Cab	++	13.75±0.41Db	++
Erythromycin	25.86±0.66Ab	+++	19.45±0.96Bc	+++	17.17±0.34Ccd	++	17.18±0.51Cb	++	13.85±0.83Db	+	15.81±0.37Cbc	++	11.97±0.93Ebc	+
Chloramphenicol	26.45±1.91Ab	+++	19.16±1.11Bc	+++	18.36±0.07BCc	+++	16.64±2.11BCb	++	15.52±1.70Cb	++	11.07±0.40Dd	+	10.17±0.41Dc	+
Streptomycin	17.59±0.50Ad	++	17.05±0.28Ac	++	14.42±1.83Bd	++	16.83±0.11Ab	++	13.63±1.40Bb	+	10.10±0.21Cd	+	9.61±0.69Cc	-
Lincomycin	22.97±0.27Abc	+++	16.23±1.28Bc	++	14.31±0.95BCDd	++	16.00±0.38BCb	++	13.97±0.77CDb	+	13.09±0.60DEcd	+	11.39±1.61Ebc	+
Tetracycline	30.14±3.15Aa	+++	26.45±1.91ABa	+++	26.45±1.91ABa	+++	21.91±3.03Ba	+++	21.91±3.03Ba	+++	20.22±2.15Ba	+++	20.22±2.15Ba	+++
Variation					**P Value**						**r**			
Type ofAntibotics(A)					<0.0001						0.194*			
Pressure (P)					<0.0001						-0.748**			
+Interaction Time (I)					<0.0001						-0.125			
A x P					<0.0001						--			
A x I					0.440						--			
P x I					0.033						--			
A x P x I					0.992						--			

a-c (→): Values shown with different letters on the same line differ from each other at the p<0.05 level, A-E (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level, ± Standard deviation,). *P* < 0.0001: Statistically too much significant, *P* =0.01: Statistically much significant, *P* =0.05: Statistically significant, **. Correlation is significant at the 0.01 level (2-tailed). ≤ 10(-): Resistant. 10- 14(+): Moderately Sensitive. 14-18 (++): Sensitive. 18≥ (+++): Ultra-sensitive. ≤ 10(-): Resistant. 10- 14 (+): Moderately Sensitive. 14-18 (++): Sensitive. 18≥ (+++): Ultra-sensitive.

Table 3. Antibiotic sensitivity of *S. thermophilus* after different temperature and duration application

Type of Antibiotics	Control		Temperature											
			0°C				-18°C				-75°C			
			1 Hour		2 Hour		1 Hour		2 Hour		1 Hour		2 Hour	
Penicilin	22.43±0.72^{Abc}	+++	21.65±0.42^{ABb}	+++	21.15±0.85^{ABb}	+++	20.68±0.55^{Bb}	+++	20.39±0.26^{Bb}	+++	18.10±0.77^{Cb}	+++	17.27±2.98^{Cbc}	++
Amoxcicilin	20.66±0.80^{Abc}	+++	20.49±0.44^{Ab}	+++	19.80±0.37^{ABbc}	+++	19.36±1.85^{ABbc}	+++	18.70±0.93^{ABbc}	+++	18.37±0.73^{ABb}	+++	17.47±1.50^{Bbc}	++
Cephalexin	19.91±0.77^{Ac}	+++	15.87±0.09^{Bc}	++	13.28±0.04^{CDd}	++	14.67±0.88^{BCde}	++	12.38±0.93^{DEef}	+	10.90±0.92^{EFc}	+	9.13±0.15^{Fd}	-
Erythromycin	23.91±2.19^{Ab}	+++	22.86±0.47^{ABb}	+++	20.50±0.06^{BCb}	+++	19.02±0.08^{CDbc}	+++	16.29±1.07^{DEcd}	++	17.36±0.51^{CDEb}	++	14.73±1.41^{Ec}	++
Chloramphenicol	20.86±1.73^{Abc}	+++	20.65±0.38^{Ab}	+++	18.89±1.08^{ABbc}	+++	19.26±1.35^{ABbc}	+++	17.16±1.40^{BCcd}	++	18.38±0.94^{ABb}	+++	14.79±0.65^{Cc}	++
Streptomycin	18.73±1.29^{Ac}	+++	15.21±0.68^{Bc}	++	14.27±1.32^{BCd}	++	12.22±0.37^{CDe}	+	10.79±0.57^{DEf}	+	10.28±0.15^{DEe}	+	9.28±0.21^{Ed}	-
Lincomycin	19.31±1.91^{Ac}	+++	17.60±0.35^{ABc}	++	16.10±1.34^{Bcd}	++	16.95±0.14^{ABcd}	++	14.88±0.78^{Bde}	++	16.80±0.05^{ABb}	++	14.79±0.92^{Bc}	++
Tetracycline	33.04±2.34^{Aa}	+++	28.38±1.92^{ABa}	+++	26.33±4.39^{BCa}	+++	26.45±1.91^{BCa}	+++	25.81±0.30^{BCa}	+++	20.89±0.56^{Ca}	+++	22.54±1.42^{BCa}	+++
Variation			**P Value**								**r**			
TypeofAntibotics(A)			<0.0001								0.168			
Temoerature (T)			<0.0001								-0.496**			
InteractionTime(I)			<0.0001								-0.114			
A x T			<0.0001								--			
A x I			0.409								--			
T x I			0.064								--			
A x T x I			0.992								--			

a-e (→): Values shown with different letters on the same line differ from each other at the p<0.05 level, A-E (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level, ± Standard deviation, *P* < 0.0001: Statistically too much significant, **. Correlation is significant at the 0.01 level (2-tailed). ≤ 10(-): Resistant. 10- 14(+): Moderately Sensitive. 14-18(++): Sensitive. 18≥ (+++): Ultra-sensitive. ≤ 10 (-): Resistant. 10- 14 (+): Moderately Sensitive. 14-18 (++): Sensitive. 18≥ (+++): Ultra-sensitive.

Table 4. Antibiotic sensitivity of *L. delbrueckii* subsp. *bulgaricus* after different temperature and duration application

Type of Antibiotics	Control		Temperature											
			0°C				-18°C				-65°C			
			1 Hour		2 Hour		1 Hour		2 Hour		1 Hour		2 Hour	
Penicilin	23.26±0.24^{Abc}	+++	22.88±0.33^{ABb}	+++	20.01±0.84^{Cab}	+++	21.04±0.53^{BCb}	+++	16.89±0.32^{Da}	++	16.36±1.62^{Db}	++	12.31±1.41^{Eab}	+
Amoxcicilin	21.84±0.13^{Ac}	+++	19.69±0.74^{ABc}	+++	16.39±0.93^{Ccd}	++	18.38±1.36^{BCbcd}	+++	13.03±1.83^{Dabc}	+	15.87±0.22^{Cb}	++	12.97±2.19^{Da}	+
Cephalexin	25.93±2.06^{Ab}	+++	22.30±1.11^{ABbc}	+++	19.89±0.91^{Bab}	+++	16.07±2.24^{Cd}	++	12.78±1.32^{CDbc}	+	14.13±1.35^{Cb}	++	9.58±1.49^{Dbc}	-
Erythromycin	25.86±0.66^{Ab}	+++	21.76±2.05^{Bbc}	+++	19.95±2.11^{BCab}	+++	17.78±0.76^{CDcd}	++	13.98±1.91^{Eabc}	+	14.82±2.26^{DEb}	++	9.91±0.18^{Fbc}	-
Chloramphenicol	26.45±1.91^{Ab}	+++	22.80±1.95^{ABb}	+++	19.60±1.49^{BCDab}	+++	20.62±0.64^{BCb}	+++	15.99±2.02^{Dab}	++	17.96±1.76^{CDab}	++	9.25±0.54^{Ec}	-
Streptomycin	17.59±0.50^{Ad}	++	16.10±0.40^{Ad}	++	13.62±1.40^{Be}	+	12.77±0.51^{Be}	+	10.70±0.93^{Cc}	+	9.92±0.88^{CDc}	-	8.85±0.32^{Dc}	-
Lincomycin	22.97±0.27^{Abc}	+++	20.37±0.37^{ABbc}	+++	17.50±1.45^{CDbc}	++	19.84±0.26^{BCbc}	+++	14.50±1.59^{Eabc}	++	16.26±1.95^{DEb}	++	10.86±0.92^{Fabc}	+
Tetracycline	30.14±3.15^{Aa}	+++	26.11±1.35^{ABa}	+++	22.28±0.78^{BCa}	+++	24.11±1.33^{BCa}	+++	16.48±1.64^{DEab}	++	20.63±1.37^{CDa}	+++	13.26±1.17^{Ea}	+
Variation			**P Value**								**r**			
Type ofAntibotics(A)			<0.0001								0.068			
Temoerature (T)			<0.0001								-0.758**			
Interaction Time(I)			0.251								-0.044			
A x T			0.501								--			
A x I			0.632								--			
T x I			0.398								--			
A x T x I			<0.0001								0.068			

a-d (→): Values shown with different letters on the same line differ from each other at the p<0.05 level, A-F (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level, ± Standard deviation, *P* < 0.0001: Statistically too much significant, **. Correlation is significant at the 0.01 level (2-tailed). ≤ 10(-): Resistant. 10- 14(+): Moderately Sensitive. 14-18(++): Sensitive. 18≥ (+++): Ultra-sensitive.

Table 5. Antibiotic sensitivity of *S. thermophilus* after different magnetic field and duration application

Type of Antibiotics	Control		Magnetic Field											
			180 µT				240 µT				300 µT			
			1 Hour		2 Hour		1 Hour		2 Hour		1 Hour		2 Hour	
Penicilin	22.43±0.72^{Abc}	+++	18.73±1.28^{Bab}	+++	14.94±0.26^{Cbc}	++	15.80±1.23^{Cabc}	++	12.83±0.56^{Dabc}	+	12.26±1.30^{Db}	+	10.01±0.25^{Eb}	+
Amoxcicilin	20.66±0.80^{Abc}	+++	17.16±1.52^{Bb}	++	14.98±0.95^{Cbc}	++	13.84±0.84^{CDcd}	+	12.01±0.65^{DEcd}	+	11.13±0.27^{EFbcd}	+	9.67±0.52^{Fbc}	-
Cephalexin	19.91±0.77^{Ac}	+++	14.24±1.12^{Bc}	++	12.29±1.29^{Ccd}	+	10.94±0.44^{CDe}	+	9.87±0.30^{DEde}	-	9.22±0.57^{DEe}	-	8.51±0.50^{Ebc}	-
Erythromycin	23.91±2.19^{Ab}	+++	19.45±0.96^{Bab}	+++	17.65±1.57^{BCab}	++	17.18±0.51^{BCa}	++	14.31±1.59^{CDab}	++	15.81±0.37^{BCDa}	++	12.37±1.53^{Da}	+
Chloramphenicol	20.86±1.73^{Abc}	+++	18.22±1.24^{ABab}	+++	16.56±1.39^{BCab}	++	14.49±1.20^{CDbcd}	++	12.29±0.47^{DEbc}	+	10.52±1.18^{Ebcd}	+	9.31±0.29^{Ebc}	-
Streptomycin	18.73±1.29^{Ac}	+++	13.34±1.00^{Bc}	+	11.36±1.66^{BCd}	+	10.80±0.99^{Ce}	+	9.04±0.39^{CDe}	-	9.98±0.15^{CDde}	-	8.01±0.33^{Dc}	-
Lincomycin	19.31±1.91^{Ac}	+++	13.76±0.54^{Bc}	+	11.85±0.52^{BCDd}	+	12.88±0.20^{BCde}	+	10.86±0.96^{CDcde}	+	11.04±0.44^{CDbcd}	+	9.35±0.44^{Dbc}	-
Tetracycline	33.04±2.34^{Aa}	+++	19.99±0.46^{Ba}	+++	17.88±0.63^{BCa}	++	16.38±1.51^{BCab}	++	14.61±1.48^{CDa}	++	11.63±1.41^{DEbc}	+	9.65±0.59^{Ebc}	-
Variation			**P Value**								**r**			
Type ofAntibotics(A)			<0.0001								0.059			
Magmetic Field (M)			<0.0001								-0.796**			
Interaction Time(I)			<0.0001								-0.141			
A x M			<0.0001								--			
A x I			0.800								--			
M x I			<0.0001								--			
A x M x I			1.000								--			

a-c (→): Values shown with different letters on the same line differ from each other at the p<0.05 level, A-F (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level, ± Standard deviation, *P* < 0.0001: Statistically too much significant, **. Correlation is significant at the 0.01 level (2-tailed). ≤ 10(-): Resistant. 10- 14(+): Moderately Sensitive. 14-18(++): Sensitive. 18≥ (+++): Ultra-sensitive.

Table 6. Antibiotic sensitivity of *L. delbrueckii* subsp. *bulgaricus* after different magnetic field and duration application

Type of Antibiotics	Control		Magnetic Field											
			180 µT				240 µT				300 µT			
			1 Hour		2 Hour		1 Hour		2 Hour		1 Hour		2 Hour	
Penicilin	23.26±0.24^{Abc}	+++	18.88±0.91^{Bb}	+++	15.88±2.21^{CDbc}	++	17.98±0.89^{BCab}	++	13.34±1.18^{Dabc}	+	13.37±1.04^{Da}	+	9.92±0.36^{Eab}	-
Amoxcicilin	21.84±0.13^{Ac}	+++	15.85±1.96^{Bb}	++	13.79±2.1^{BC4cd}	+	14.77±1.39^{Bbc}	+	11.11±0.61^{CDbc}	+	11.12±0.85^{CDa}	+	10.15±0.13^{Da}	+
Cephalexin	25.93±2.06^{Ab}	+++	23.47±1.32^{ABa}	+++	19.54±1.15^{BCab}	+++	17.75±2.24^{CDab}	++	14.60±3.14^{DEab}	++	10.72±0.77^{EFa}	+	8.84±0.51^{Fbc}	-
Erythromycin	25.86±0.66^{Ab}	+++	23.24±1.24^{Aa}	+++	18.94±0.69^{Bab}	+++	16.54±1.61^{Bb}	++	11.57±1.55^{Cbc}	++	11.60±2.20^{Ca}	+	9.70±0.46^{Cab}	-
Chloramphenicol	26.45±1.91^{Ab}	+++	25.39±1.72^{Aa}	+++	21.9±2.37^{ABa}	+++	21.72±0.62^{ABa}	+++	16.41±1.54^{BCa}	++	15.18±5.16^{CDa}	++	9.80±0.29^{Dab}	-
Streptomycin	17.59±0.50^{Ad}	++	14.94±2.23^{Ab}	++	10.93±0.53^{Bd}	+	11.60±2.13^{Bc}	+	9.97±0.15^{BCc}	-	10.79±1.01^{BCa}	+	7.94±0.66^{Cc}	-
Lincomycin	22.97±0.27^{Abc}	+++	19.13±1.91^{Bb}	+++	15.80±2.44^{BCbc}	++	14.74±2.36^{CDbc}	++	11.75±1.43^{DEbc}	+	11.56±1.12^{DEa}	+	9.38±0.38^{Eab}	-
Tetracycline	30.14±3.15^{Aa}	+++	24.02±2.19^{Ba}	+++	19.46±1.47^{Cab}	+++	16.61±1.01^{CDb}	++	13.74±0.80^{DEabc}	++	10.99±0.11^{Ea}	+	9.89±0.46^{Eab}	-
Variation			**P Value**								**r**			
Type ofAntibotics(A)			<0.0001								0.032			
Magmetic Field (M)			<0.0001								-0.829**			
Interaction Time(I)			0.912								0.004			
A x M			<0.0001								--			
A x I			0.684								--			
M x I			0.579								--			
A x M x I			0.699								--			

a-c (→): Values shown with different letters on the same line differ from each other at the p<0.05 level, A-F (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level, ± Standard deviation, *P* < 0.0001: Statistically too much significant, **. Correlation is significant at the 0.01 level (2-tailed). ≤ 10(-): Resistant. 10- 14(+): Moderately Sensitive. 14-18(++): Sensitive. 18≥ (+++): Ultra-sensitive.

CONCLUSION

This study aimed to reveal the changes that might occur in the susceptibilities of 2 yogurt bacteria types to antibiotics under 2 different durations and 3 different stress conditions and present new approaches to reduce issues arising from using milk with antibiotics in the fermented milk industry. The study found that as the severity and application duration of the stress conditions increased, the antibiotic susceptibility in the bacteria decreased, and resistance to certain antibiotic types developed. In the conclusion of the 3 different stress applications, it was determined that the bacteria had the highest resistance to the antibiotics in the magnetic field applications. In these 3 different stress applications, *S. thermophilus* showed the highest resistance to lincomycin, cephalexin, and streptomycin, and *L. delbrueckii* subsp. *bulgaricus* developed resistance to streptomycin, erythromycin, and chloramphenicol. Of the 2 yogurt bacteria, *L. delbrueckii* subsp. *bulgaricus* developed resistance to more antibiotics than *S. thermophilus* after the stress applications; in addition, the developed resistance was more substantial than that of *S. thermophilus*.

Beta-lactam antibiotics (penicillin, cephalosporins, etc.), mainly used in treating diseases such as mastitis, pass into milk and cause severe problems—from quality loss in the end-product to an inability to produce a product. Since the stress applications on the starter cultures used in production decrease the susceptibility of these bacteria to antibiotics, it is believed that potential issues that might arise from the antibiotic content can be drastically reduced. The relationship between stress applications and antibiotic residue in milk must be further examined in similar studies.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: Authors are equal to the article declared that they have contributed significantly.

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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