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# Kefir's Hidden Arsenal: Examining the Effect of Lactobacilli Supernatant on Antibiotic Resistance Genes and Virulence in Salmonella Typhimurium

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**Abstract**:
Salmonella, a prominent foodborne pathogen, poses significant health risks, causing both intestinal and extra-intestinal infections. Recognizing the potential of lactobacilli as probiotics due to their ability to produce substances inhibiting multidrug-resistant bacteria, this study aimed to assess antibiotic resistance, pathogenic gene frequency, antibacterial effects of lactobacillus supernatant from kefir, and its impact on resistance and pathogenicity gene expression.
In Tehran hospitals, 150 isolates from 240 clinical samples were collected and identified as Salmonella typhimurium using biochemical and serotype tests. Antibiotic sensitivity was assessed, and the frequencies of antibiotic resistance genes (tetA, tetB, and floR) and pathogenicity genes (sip, spvC, and invA) were investigated. Lactobacilli from kefir were isolated, and the minimum inhibitory concentration of lactobacillus supernatant was determined. The relationship between supernatant treatment and tetA and sip gene expression was examined using Real-time PCR.
Results revealed 38% of strains as Salmonella typhimurium serotype, displaying high resistance to ampicillin, tetracycline, and nitrofurantoin. Pathogenicity genes invA and sip exhibited high frequencies of 100% and 70.2%, respectively. Lactobacillus supernatant showed an MIC of 80 µg/ml, effectively reducing tetA and sip gene expression by 42.2% and 55.7%, respectively.
In conclusion, the study underscores the high antibiotic resistance in Salmonella typhimurium and suggests Meropenem, Trimethoprim Sulfamethoxazole, and Ampicillin-Sulbactam as effective treatments. Moreover, lactobacillus supernatant demonstrated significant potential against Salmonella typhimurium, highlighting lactobacilli as promising probiotics. This health-oriented strategy presents a viable solution for treating Salmonella infections and preventing their spread.

## INTRODUCTION

Enterobacteriaceae family is a diverse and extensive group of Gram-negative bacilli encompassing a wide range of species and generaThe most important pathogenic genera in this family that affect humans include Escherichiaspp , Klebsiella spp, Salmonellaspp , Shigella spp , and Yersinia spp(1). This large and heterogeneous family produces a set of antigenic structures and virulence factors that cause disease in humans and animals (2). Salmonella species are regarded as common foodborne infections due to their vast range of animal reservoirs, causing considerable global public health issues with significant economic repercussions. These gram-negative bacteria are rod-shaped, small, and lack capsules, but have surrounding flagella that measure 4.5-2.5 microns. Over 2700 unique Salmonella species have been found to date in diverse places throughout the world (3, 4).

It is estimated that 16 million cases of typhoid fever, 1.3 million cases of gastroenteritis, and 3 million deaths worldwide are attributed to salmonella. It is estimated that 16 million cases of typhoid fever, 1.3 million cases of gastroenteritis, and 3 million deaths worldwide are attributed to salmonella. Studies have demonstrated that poultry serves as a significant reservoir of human salmonellosis. This is due to the bacterium's ability to

colonize the digestive system and cloaca of poultry, which are important sites for salmonella colonization. (5-7).

Salmonella enteritidis and Salmonella typhimurium have been reported as two common serotypes isolated from different cases. Infection caused by these two requires treatment or even leads to hospitalization (8). The rapid emergence of antimicrobial resistance by microbial pathogens is a threat to public health. The emergence of microbial resistance is not a new phenomenon and the production of new antibiotics has become a challenge in disease control in poor and developing countries. In 2014, the World Health Organization (WHO) named antibiotic resistance as a major global threat. This organization has reported the increase of drug resistance in all parts of the world by reviewing the statistics of 114 countries (9). Salmonella species employ genes and pathogenic determinants for the invasion of host cells and the initiation of pathogenic processes. The invA gene is responsible for encoding the invasive protein invA, which plays a pivotal role in attacking intestinal epithelial cells, ultimately resulting in the onset of the disease. Additionally, this gene contributes to the transportation of the invE protein. The invA gene facilitates the ingress of bacteria into epithelial cells and exhibits structural similarities to genes associated with flagella biosynthesis. Furthermore, the SipA protein assumes a crucial function in actin polymerization. Salmonella employs the type 3 secretion system (T3SS) to inject the 12 effector proteins encoded by the pathogenicity island 1 (SPI-1) into the host cell. These proteins, likely the first to be released by SipC and SipA, include SipA and SipC. The SipA and SipC proteins induce membrane roughness, promote invasion, and contribute to stabilization through their direct interaction with the actin cytoskeleton. Additionally, they play a regulatory role in actin movement and dynamics (10-12).

According to different research and publications, antibiotic resistance in Salmonella is becoming an increasing concern. Antibiotic-resistant nontyphoidal Salmonella infections are on the rise, according to the Centers for Disease Control and Prevention (CDC), with some strains resistant to key medicines such as ciprofloxacin, azithromycin, and ceftriaxone. Salmonella strains have also been reported to be resistant to a variety of antibiotics, including streptomycin, gentamicin, and sulfadimethoxine. Furthermore, a strain of extremely drug-resistant (XDR) Salmonella typhi has evolved that is resistant to all antibiotic classes except two (13). This trend emphasizes the necessity of combating antibiotic resistance in Salmonella with comprehensive tactics, such as the investigation of alternate control measures and the identification of efflux pumps, regulators, and inhibitors to tackle multidrug resistance.

Probiotics are live microorganisms that are similar to the beneficial microorganisms found in the human gut (14). Probiotics have been demonstrated to effectively alleviate symptoms of lactose intolerance by breaking down lactose with the production of the beta-D-glucosidase enzyme, thereby, preventing or minimizing occurrences of diarrheal diseases. Additionally, probiotics play a role in preventing and managing allergies, as evidenced by studies indicating that probiotics containing Lactobacillus GG may reduce the prevalence of atopic eczema in later stages of life. Beyond these applications, probiotics exhibit important properties such as anti-genotoxic, anti-mutagenic, and anti-cancer effects, along with a reduction in the production of carcinogenic or toxic metabolites. Epidemiological studies further support the use of probiotics in reducing the incidence of colon cancer through various mechanisms (15-17).

Kefir is a fermented milk beverage made by lactic acid bacteria, acetic acid bacteria, and yeast. Lactobacilli are among the most often encountered bacteria in kefir (18, 19). These microorganisms have been shown to reduce bacteria pathogenicity and hinder several virulence factors (20). The aim of this study is to investigate the impactof Lactobacilli Supernatant on antibiotic resistance genes and virulence in Salmonella Typhimurium.

## MATERIALS AND METHODS

This research was descriptive-cross-sectional.

**Table 1.** The function of virulence genes in the pathogenic process of Salmonella bacteria

| Gene | Gene function and role |
|---|---|
| InvA | Role in the invasion and invasion of intestinal mucosa and epithelial tissue |
| SipA | Actin polymerization, role in bacterial motility and spread |
| Spv | 1. Helping the survival of bacteria inside the host cells<br>2. Helping the growth and reproduction of the pathogen in the host's body and in extra-intestinal places<br>3. Helping the systemic spread of the pathogen in the host's body (systemic infection) |

240 stool samples from patients with diarrhea were collected from Imam Khomeini, Shahada Tajrish and Luqman Hospitals during the period of 1401 to 1402 in Tehran and were transferred to the laboratory at 4°C using the appropriate method. The strains were cultured in TSB medium and then it was transferred to EMB and McConkey agar medium, warm staining was done from suspicious colonies and finally they were subjected to biochemical tests such as Oxidase, ONPG, MR-VP for final confirmation (21). For drug sensitivity determination, Mueller Hinton Agar culture medium used in performing the disk diffusion test by McFarland half solution for 20-24 hours (22). Based on the Kaufman-White method, the serotypes of Salmonella isolates can be identified, this method is based on the agglutination of bacteria with specific somatic (O) and flagella (H) antisera (23). After drug resistance, in order to check the frequency of antibiotic resistance genes and pathogenicity genes, molecular tests were performed. The existence of these genes was investigated by multiplex PCR against the antibiotic resistance genes tetA, tetG, and floR and the pathogenicity genes sip, spvC, and invA (24) (Table 2).

The bacteria isolated from kefir were cultured and their extracts were obtained. In this research, microscopic and physicochemical methods were used for the initial identification of the obtained strain (25). The effects of these extracts on the selected strain were performed using the microdilution method, and then the effect of this treatment on the expression of the pathogenic gene sip and the resistance gene to the antibiotic tetracycline ( test) was done using Real-time PCR method. The results are interpreted using the ΔCT method. The quantification of the augmentation in gene copy numbers of specific genes in the presence of nanoparticles was computed by dividing the number of gene copies post-treatment by the number of copies pre-treatment. The assessment of resistance is contingent upon this observed augmentation, a parameter that varies according to the specific antimicrobial substance under consideration.

All the methods were presented in supplementary file.

## RESULTS

In this research, 150 isolates isolated from people suspected of intestinal disease from Imam Khomeini, Shahada Tajrish, and Luqman hospitals in Tehran were prepared and then subjected to morphological, biochemical, and antibiotic resistance studies by disc diffusion method, and of these, 57 samples The strain that was known as Salmonella typhimurium. (Table3). The frequency of resistance gene to tetracycline (tetA/B) and florfenicol (floR) antibiotics were checked by PCR method. Then the lactobacillus isolated from the cultured kefir and its supernatant were obtained and treated against the selected strain, and finally, changes in the expression of the pathogenicity gene (sip) and the tetracycline antibiotic resistance gene (tetA) were determined using the Realtime PCR method (Figure 1-2).

The Salmonella strains in this study showed high

**Table 2.** Sequences of primers used in this research

| desired gene | Primer sequence | PCR product size |
|---|---|---|
| floR | AACCCGCCCTCTGGATCAAGTCAA<br>CAAATCACGGGCCACGCTGTATC | 548 |
| tetA | GCT ACA TCC TGC TTG CCT TC<br>CAT AGA TCG CCG TGA AGA GG | 210 |
| tetB | TTG GTT AGG GGC AAG TTT TG<br>GTA ATG GGC CAA TAA CAC CG | 659 |
| invA | CGCGGCCCGATTTTCTCTGGA<br>AATGCGGGGATCTGGGCGACAAG | 321 |
| sipB/C | ACAGCAAAATGCGGATGCTT<br>GCGCGCTCAGTGTAGGACTC | 232 |
| spvC | ACTCCTTGCACAACCAAATGCGGA<br>TGTCTTCTGCATTTCGCCACCATCA | 424 |

**Table 3.** Information of the strains isolated

| Number of strains | | | |
|---|---|---|---|
| **Total number of 150 pieces** | **Female (total number 58)** | **Male (total number 92)** | **Serotypes** |
| 57 (38 percent) | 21 | 36 | *Salmonella typhimurium* |
| 93 (62 percent) | 37 | 56 | *Salmonella enteritidis* |

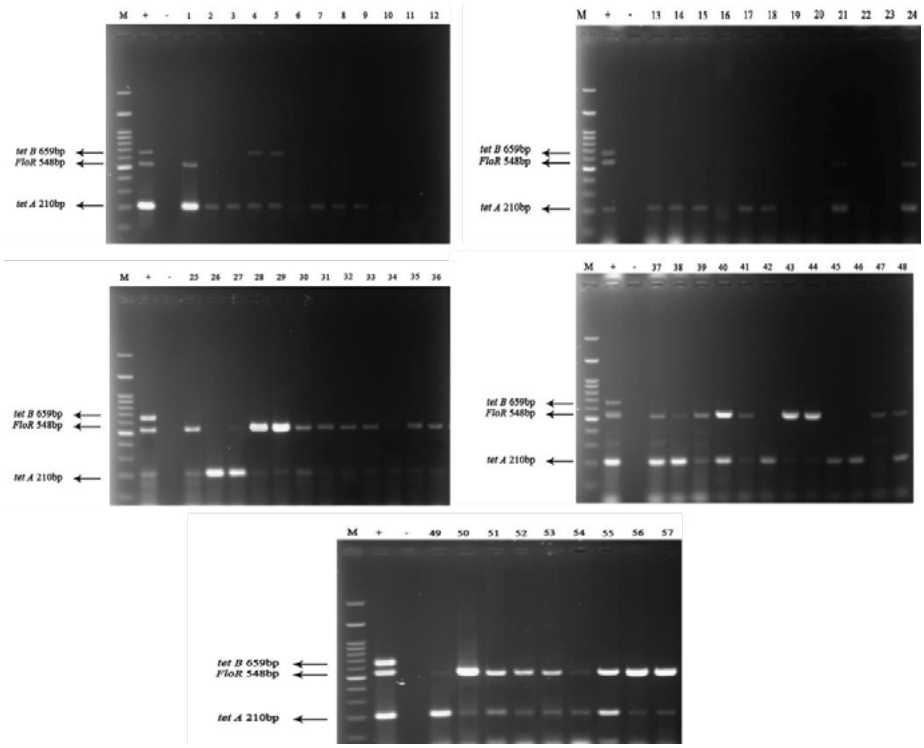


**Fig1.** Electrophoresis results of PCR products for tetA, tetB and floR genes, for isolates 1 to 57, M gene marker, + positive control (Salmonella typhimurium ATCC 14028 strain) and - negative control (distilled water).

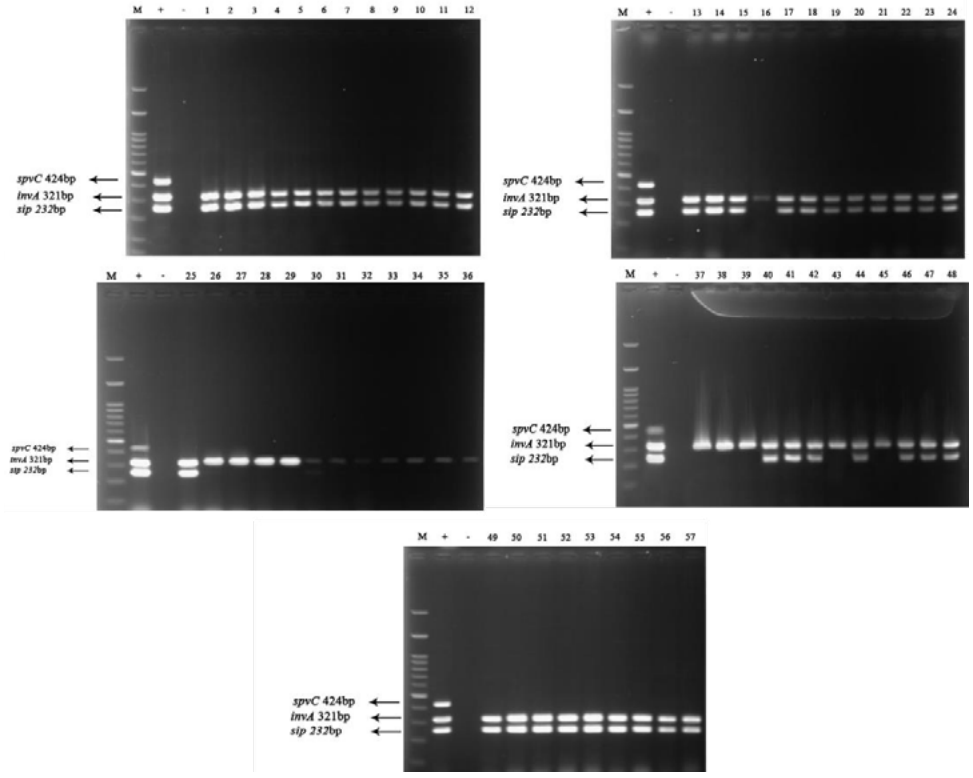


**Fig 2**. Electrophoresis results of PCR products for sip, invA and spvC genes, for isolates 1 to 57, M gene marker, + positive control (Salmonella typhimurium ATCC 14028 strain) and - negative control (distilled water).

antibiotic resistance, so that compared to ampicillin (100%), tetracycline (100%) and nitrofuranthion (84.2 to 100%) the most showed resistance and compared to meropenem (1.1 to 1.8 percent), trimethoprim sulfamethoxazole (1.8 to 2.2 percent) and ampicillin-sulbactam (3.5 to 4.3 percent) Showed. As can be

seen, Salmonella Typhimurium serovar also showed high resistance to different classes of antibiotics, showing the highest resistance to ampicillin (100%), tetracycline (100%) and nitrofuranthion (84.2%). They also showed sensitivity to the antibiotic meropenem (1.8%), trimethoprim-sulfamethoxarol (1.8%), and ampicillin-sulbactam (3.5%)(Table 4).

The genetic data results indicate that the tetracycline resistance gene was most prevalent, with 86% of the strains carrying the gene. Additionally, a high frequency of the florfenicol resistance gene was observed, with 56.1% (32) of the strains containing this None of the strains contained all three studied genes, but 54.4% (31) of the strains contained both tetracycline and florfenical resistance genes (Table 5).

The results of the genetic data show that the highest frequency of the pathogenicity gene in the studied strains was observed for the invA gene and all the strains (100%) had this gene, the frequency of the sip gene was 72.2% (40) and None of the strains (0%) had the spvC gene (Table 6).

The results showed that bacterial growth was inhibited at a concentration of 160 µg/ml of the supernatant, and this concentration was considered as MIC, and a concentration of 80 µg/l was reported as sub-MIC (Table 7).

Results of tetA gene expression change in pre and post treatment with Lactobacillus supernatant by Real time PCR method evaluated by melting curves and ΔΔCT Method. The expression level, which is a measure

**Table 4.** The results of the frequency of antibiotic resistance of isolates

| | **SALMONELLA ENTERITIDIS (93)** | | **SALMONELLA TYPHIMURIUM(57)** | | |
|---|---|---|---|---|---|
| Antibiotics | Percent | resistant | Percent | Number | **DENSITY** |
| Ampicillin | % 100 | 93 | % 100 | 57 | **10** |
| Ampicillin-sulbactam | % 4/3 | 4 | % 3/5 | 2 | **100-10** |
| Amoxicillin-clavulanic acid | % 69/9 | 65 | % 54/4 | 31 | **20-10** |
| Cefataxime | % 51/8 | 54 | % 31/6 | 18 | **30** |
| Amy Panam | % 25/8 | 24 | % 36/8 | 21 | **10** |
| Gentamicin | % 6/5 | 6 | % 5/3 | 3 | **10** |
| Tetracycline | % 100 | 93 | % 100 | 57 | **30** |
| Ciprofloxacin | % 22/6 | 21 | % 14 | 8 | **5** |
| Nalidixic acid | % 38/7 | 36 | % 43/9 | 25 | **30** |
| Trimethoprim Sulfomethoxazole | % 2/2 | 2 | % 1/8 | 1 | **25** |
| Chloramphenicol | % 22/6 | 21 | % 7 | 4 | **30** |
| Azithromycin | % 25/8 | 24 | % 26/3 | 15 | **300** |
| Nitrofuranthion | % 100 | 193 | % 84/2 | 48 | **300** |
| Meropenem | % 1/1 | 1 | % 1/8 | 1 | **2** |

**Table 5**. Frequency of antibiotic resistance genes in Salmonella typhimurium strains

| **PERCENT** | **NUMBER** | **GENE NAME** |
|---|---|---|
| % 86 | 49 | **TETA** |
| % 1/8 | 1 | **TETB** |
| % 56/1 | 32 | **FLOR** |
| % 0 | 0 | **ALL GENES** |
| % 54/4 | 31 | **TET+FLOR** |

**Table 6.** Frequency of sip, invA and spvC pathogenicity genes in Salmonella Typhimurium strains

| PERCENT | NUMBER | GENE NAME |
|---|---|---|
| % 0 | 0 | SPVC |
| % 100 | 57 | INVA |
| % 70/2 | 40 | SIP |

**Table 7.** The results of the minimum inhibitory concentration of treatment with lactobacillus supernatant against the selected strain

| CONCENTRATION OF TUNGSTEN OXIDE NANOPARTICLES (MICROGRAMS/ML) | SALMONLATIFI MORIUM |
|---|---|
| 160 | **MIC** |
| 80 | **SUB MIC** |
| 2560 | **INITIAL CONCENTRATION** |

of ΔΔCT, is obtained using the calculations of cycle thresholds (Ct) finally, to calculate the fold change, the expression of the treated gene (0.579012607) should be subtracted from the control (1.00126) ) to be calculated, which was calculated as 0.578 in this research, which shows that the expression of resistance genes decreased by 42.2% during the treatment. As shown in Table 6-4, the statistical analysis of the obtained results was done and the relationship between the treatment and the obtained results was tested under the condition of $P<0.05$ and the results were confirmed. (Table 8) In order to calculate the average relative expression, the average of ΔΔCTs was first taken and the result was calculated with the formula of relative expression (Table 9) (Figure 3).

Results of sip gene expression change in pre and post treatment with Lactobacillus supernatant by Realtime PCR method evaluated by melting curves and ΔΔCT Method, the expression level, which is a measure of ΔΔCT, to calculate the fold change, the expression of the treated gene (0.443943) should be subtracted from the control (1.00098). in this study it was calculated as 0.443508, which shows that the expression of resistance genes decreased by 55.7% during the treatment. the statistical analysis of the obtained results was done and the relationship between the treatment and the obtained results was tested under the condition of $P<0.05$ and the results were confirmed. In order to calculate the average relative expression, the average of ΔΔCTs was first taken and the result was calculated with the formula of relative expression (Table 10, 11) (Figure 3).

## DISCUSSION

The research findings indicate that Salmonella typhimurium constitutes a significant portion (38%) of isolates in individuals with intestinal infections. Notably, 63% of these isolates are found in men, emphasizing the prevalence of this strain in the male population. Antibiotic resistance patterns reveal high resistance to ampicillin, tetracycline, and nitrofuranthion, with multidrug resistance observed.

**Table 8.** Mean relative expression for tetA gene

| STANDARD DEVIATION (SD) | MEAN RELATIVE EXPRESSION | |
|---|---|---|
| **0/061826** | **1** | Control |
| **0/01853** | 0/579012607 | treatment |

**Table 9.** Mean relative expression for sip gene

| STANDARD DEVIATION (SD) | MEAN RELATIVE EXPRESSION | |
|---|---|---|
| **0/0537** | **1** | Control |
| **0/04601** | 0/44237 | treatment |

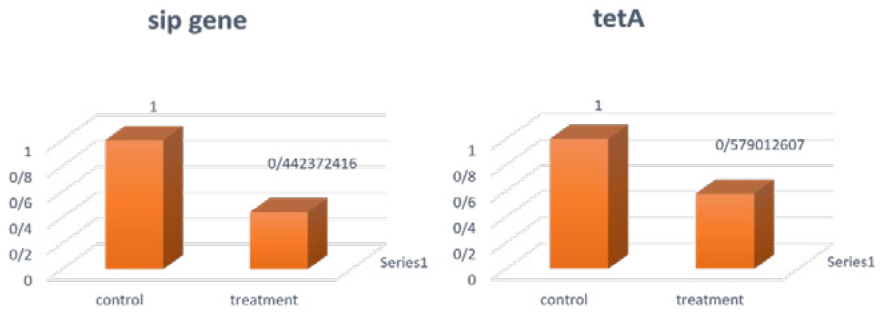


**Fig 3.** Mean relative expression plot for tetA and sip gene

**Table 10.** Calculation of relative expression and statistical analysis of data obtained from Real-time PCR reaction for tetA gene

| House (16S rRNA) | | | |
|---|---|---|---|
| **Name** | **CT** | **CT** | **Average ( CT )** |
| Treat | 25.71 | 25.53 | 25.62 |
| Non-Treat | 25.91 | 25.88 | 25.895 |
| **Gene (*tetA*)** | | | |
| **Name** | **CT** | **CT** | **Average ( CT )** |
| Sample 1 Treat | 26.29 | 26.23 | 26.26 |
| Sample 2 Treat | 26.41 | 26.37 | 26.39 |
| Sample 3 Treat | 26.16 | 26.09 | 26.125 |
| Sample 1 Non-Treat | 25.89 | 25.63 | 25.76 |
| Sample 2 Non-Treat | 25.87 | 25.78 | 25.825 |
| Sample 3 Non-Treat | 25.61 | 25.69 | 25.65 |
| **ΔΔCT Method** | | | |
| **Name** | **ΔCT** | **ΔΔCT** | **Fold change** |
| Sample 1 Treat | 0.64 | 0.775 | 0.584388624 |
| Sample 2 Treat | 0.77 | 0.84 | 0.558643569 |
| Sample 3 Treat | 0.505 | 0.75 | 0.594603558 |
| Sample 1 Non-Treat | -0.135 | 0.015 | |
| Sample 2 Non-Treat | -0.07 | 0.08 | |
| Sample 3 Non-Treat | -0.245 | -0.095 | |
| **Avg Fold change** | 0.5792119 | | |
| **T-Test** | | | |
| **P-value** | | **Significant or Not** | |

Meropenem, trimethoprim-centosulfamethoxarol, and amoxicillin-sulbactam are suggested for treatment. Genetic analysis indicates a high frequency of tetracycline resistance genes and pathogenicity genes, particularly invA. The inhibitory potential of lactobacillus supernatant against Salmonella is demonstrated, leading to decreased expression of tetracycline resistance and sip pathogenicity genes.

Comparing with other studies, Nosrati et al. (2012) found a high prevalence of Salmonella typhimurium and Salmonella enteritidis in food samples, emphasizing the risk from food sources in salmonella infections (26). Nazari Moghadam et al. (2023) aimed to investigate the prevalence of virulent antibiotic-resistant Salmonella spp. strains in Iranian poultry markets, highlighting the importance of monitoring this pathogen in food sources (27). Ribeiro et al. (2011) observed an increase in antimicrobial resistance among Salmonella Enterica, emphasizing the impact on public health (28). Dantas et al. (2020) highlighted the persistence and pathogenic potential of Salmonella in poultry slaughterhouse, indicating resistance to tetracycline and the role of biofilm production (29). Gomez et al. (2022) underscored the resistance of Salmonella isolates in poultry and pork to various antimicrobials, emphasizing the need for control in

**Table 11.** Calculation of relative expression and statistical analysis of data obtained from Real-time PCR reaction for tetA gene

| House (16S rRNA) | | | |
|---|---|---|---|
| **Name** | **CT** | **CT** | **Average ( CT )** |
| Treat | 18.74 | 18.42 | 18.58 |
| Non-Treat | 18.64 | 18.88 | 18.76 |

| Gene (*sip*) | | | |
|---|---|---|---|
| **Name** | **CT** | **CT** | **Average ( CT )** |
| Sample 1 Treat | 19.71 | 19.85 | 19.78 |
| Sample 2 Treat | 20.33 | 20.11 | 20.22 |
| Sample 3 Treat | 20.08 | 19.87 | 19.975 |
| | | | |
| Sample 1 Non-Treat | 18.97 | 18.91 | 18.94 |
| Sample 2 Non-Treat | 19.03 | 19.14 | 19.085 |
| Sample 3 Non-Treat | 18.85 | 19.07 | 18.96 |

| ΔΔCT Method | | | |
|---|---|---|---|
| **Name** | **ΔCT** | **ΔΔCT** | **Fold change** |
| Sample 1 Treat | 1.2 | 1.02 | 0.493116352 |
| Sample 2 Treat | 1.64 | 1.315 | 0.401925495 |
| Sample 3 Treat | 1.395 | 1.195 | 0.436786448 |
| | | | |
| Sample 1 Non-Treat | 0.18 | -0.055 | |
| Sample 2 Non-Treat | 0.325 | 0.09 | |
| Sample 3 Non-Treat | 0.2 | -0.035 | |
| **Avg Fold change** | 0.4439428 | | |

| T-Test | |
|---|---|
| **P-value** | **Significant or Not** |
| 0.001127783 | Significant |

meat production chains (30).

The studies by Golowczyc et al. in 2007 and 2008 emphasized the inhibitory power of Lactobacillus kefir strains against Salmonella, suggesting their potential as probiotics (31, 32). Jr. et al. (2018) demonstrated the probiotic potential of Lactobacillus deliverurans strain Z1 in protecting mice from Salmonella infection (33).

The increase in antibiotic resistance in Salmonella has led to the exploration of alternative approaches to control and prevent enteric bacterial infections. Studies have highlighted the potential role of probiotics, particularly Lactobacillus strains, including Lactobacillus plantarum, Lactobacillus salivarius, Lactobacillus amylovorus, and Lactobacillus kefiri, to protect against Salmonella infection through interference with its growth and virulence properties (34, 35). The antagonistic activity of Lactobacillus strains has been shown to inhibit the growth of Salmonella typhimurium in vitro, with significant growth inhibition rates observed. The inhibitory effects are attributed to various factors, including the production of antimicrobial metabolites such as lactic acid and bacteriocins (36). Lactobacillus strains have been found to exhibit adhesion capacities, auto aggregation, and coaggregation with Salmonella, leading to decreased adherence and invasion of host cells by Salmonella (32). Additionally, the production of antimicrobial metabolites, including lactic acid and bacteriocins, contributes to the inhibition of Salmonella growth and virulence properties.Additionally, research has shown that Salmonella spp. isolated from food sources exhibit resistance to various antibiotics, emphasizing the need for alternative control measures (13, 37).

In the context of antibiotic resistance, the tetA gene, which confers resistance to tetracycline, and the sip gene, associated with Salmonella pathogenicity, have been subjects of interest. The effect of kefir lactocacillus in preventing the expression of these genes, as well as its impact on antibiotic resistance

in Salmonella, presents a promising avenue for further research and potential application in infection control and prevention. Furthermore, the diversity of antimicrobial resistance genes in kefir and yogurt, including antibiotic target protection and antibiotic efflux mechanisms, has been investigated, shedding light on the complex interplay between probiotics and antibiotic resistance (35).

In conclusion, Salmonella typhimurium poses a considerable risk in causing intestinal infections, with antibiotic resistance and multidrug resistance being major concerns. The use of alternative antibiotics and the potential of probiotic interventions, particularly with lactobacillus strains, are suggested for effective treatment and prevention. The comparison with other studies emphasizes the widespread prevalence of Salmonella strains and the urgent need for control measures in various sources, including food, animals, and the environment. The interplay between antibiotic resistance in Salmonella, the role of specific genes such as tetA and sip, and the potential of kefir lactocacillus in preventing antibiotic resistance and pathogenicity gene expression represents a dynamic and evolving area of study. Further research in this field holds promise for the development of innovative strategies to address antibiotic resistance and enhance infection control and prevention measures.

*Ethical considerations*

In this research, sampling was not done directly from the patient, therefore, there is no interference in the process of diagnosis and treatment of the patient.

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