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Research Article

Preliminary investigation into the impact of food-related stressors on the antibiotic susceptibility of *Salmonella* Typhi isolated from food sources

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Abstract

Salmonella is responsible for causing significant morbidity and mortality in both humans and livestock annually. The development of antibiotic resistance in these bacteria is attributed to their exposure to various environmental stresses. Given the increase in antibiotic resistance in *Salmonella*, this study aimed to investigate the simultaneous effects of food-related stresses on the antibiotic susceptibility pattern of *Salmonella* Typhi. The stresses included osmotic pressure (1 and 4%), pH (7.3 and 5), heat (45 and 55 °C), cold (4 and 8 °C), and freezing (24 and 96 h). Changes in the antibiotic susceptibility patterns of *S.* Typhi were investigated using the disk diffusion method. The results showed that osmotic pressure and freezing time had the greatest effects on antibiotic susceptibility. Different stresses have different effects on the antibiotic susceptibility patterns of *S.* Typhi. The results of this study can be used as a basis for further research in this area.

Keywords: Animal-origin food, Antibiotic resistance, Environmental conditions, Food stresses.

Introduction

Foodborne pathogenic bacteria represent a critical concern in the domains of public health, veterinary medicine, food hygiene and safety, and human foodborne infections (Di Stasi et al., 2025). Studies on changes in the phenotypic and genotypic characteristics of foodborne pathogens are among the most important aspects of public health (Kaboudari et al., 2022). Among foodborne pathogens, *Salmonella* strains are one of the most important threats to food hygiene, public health, and veterinary medicine. The most important strains isolated from food are *S.* Enteritidis and *S.* Typhimurium (Manafi et al., 2020). However, in recent years, *S.* Typhi has been isolated from certain foods and water sources. In addition to causing food poisoning, *S.* Typhi is one of the most important causes of infectious diseases, the most important of which is typhoid fever (Jahan et al., 2022; Orji et al., 2005).

Salmonella strains are among the most important foodborne pathogenic bacteria that acquire different characteristics under the influence of different environmental conditions (Kaboudari & Aliakbarlu, 2025). *Salmonella* strains respond to different environmental conditions, such as different temperatures, acidic conditions, and osmotic

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pressures. These reactions cause changes in bacterial kinetics and function (Kang & Seo, 2020). A significant and frequently debated alteration pertains to the level of susceptibility to antimicrobial agents and antibiotics, which holds substantial importance in the context of food hygiene and safety (Al-Nabulsi et al., 2015).

Antibiotic resistance in foodborne pathogens is one of most important public health issues (Kaboudari et al., 2024a). Recent studies have shown that many foodborne pathogens, including *S.* Typhi, express genes associated with increased antibiotic resistance, which could be a major alarm for One Health goals (Pervaiz et al., 2025). Foodborne pathogens in different food environments that contain different nutrients, water activity conditions, a wide range of acidity and osmotic pressure, and the presence of antimicrobial compounds undergo changes in sensitivity or resistance to antibiotics in response to these conditions (Friedman, 2015). Studying the mechanism of antibiotic resistance in foodborne pathogens is an important factor in their control and inhibition in the human food supply chain (McMahon et al., 2007). Among foodborne pathogens, *S*. Typhi is one of the most important antibiotic-resistant pathogens in the United States (CDC, 2015). Studies have also shown that these food-borne bacteria in Iran have become resistant to several widely used broad-spectrum antibiotics (Manafi et al., 2020). Based on the studies conducted and the importance of antibiotic resistance in *S.* Typhi, in addition to the effect of conditions, stress, and various food-related factors on changes in antibiotic susceptibility, in this study, the single and combined effects of food-related stresses (temperature, pH, and osmotic pressure) on changes in antibiotic susceptibility/resistance in *S.* Typhi isolates were investigated.

Materials and Methods

Bacterial serotypes and cocktails

Two previously isolated *Salmonella* strains belonging to two serotypes of *Salmonella enterica* serovar Typhi (Manafi et al., 2020) were used in this study (serotypes 32sh and 49b). The bacterial serotypes were stored at -20 °C in 30% glycerol. Before the experiments, the bacteria were re-cultured twice in TSB (Trypticase soy broth (Quelab, Montréal, Québec, Canada) and incubated at 37 °C for 20 h.

Table 1. Factorial design used to evaluate the effects of stress factors on the antibiotic resistance of *Salmonella* Typhi

Runs	Factors				
	A: NaCl (%)	B: pH	C: Heat (˚C)	D: Cold (˚C)	E: Freezing time (h)
1	1	7.3	45	4	24
2	1	7.3	55	8	24
3	4	5	45	4	24
4	1	5	45	8	24
5	1	5	45	4	96
6	4	7.3	55	4	24
7	4	7.3	55	8	96
8	4	5	55	8	24
9	1	7.3	55	4	96
10	4	7.3	45	4	96
11	4	5	45	8	96
12	1	7.3	45	8	96
13	4	7.3	45	8	24
14	4	5	55	4	96
15	1	5	55	8	96
16	1	5	55	4	24

Bacterial cocktails of two *Salmonella* serotypes were used to investigate the pattern of changes in antibiotic resistance in *Salmonella* Typhi. A previously described method was used to prepare a bacterial cocktail of each strain (Lastra-Vargas et al., 2020). Each strain in the exponential growth phase was cultured in 10 mL of TSB and incubated at 37 °C for 20 h. The culture media were centrifuged at 4000 rpm for 10 min, and the bacterial pellet was washed twice with phosphate-buffered saline (PBS). The pellet was then resuspended in PBS, and the bacterial count was adjusted to 1 × 10^8 CFU/mL. To prepare the cocktail, 1 mL of each suspension was mixed with 1 ml of the same strain suspension. The prepared cocktails were cultured in TSB twice (37 °C for 20 h each). The second culture was used as a stock culture and stored in a cryotube (-80 °C) for subsequent experiments.

Table 2. Regression coefficients of the model for the antibiotic resistance response of *Salmonella* Typhi

Main and Interaction Factors	Antibiotics			
	C	**GM**	**CRO**	**ST**
A	+0.72*	NI	+0.78*	NI
B	+0.28*	NI	NI	NI
C	NI	-0.54	-0.66*	-0.31*
D	NI	NI	NI	NI
E	+5.12*	NI	+1.36*	NI
AB	-0.47*	NI	NI	NI
AC	+0.79*	NI	NI	NI
AD	-0.2*	+0.56	NI	NI
AE	+1.57*	NI	-0.62*	+0.56*
BC	NI	NI	NI	-0.28*
BD	NI	NI	-0.78*	NI
BE	+1.72*	NI	NI	+.025*
CD	-1.16*	NI	NI	-0.40*
CE	NI	NI	NI	NI
DE	NI	NI	-0.64*	NI
R^2	0.99	0.38	0.90	0.88
Adjusted R^2	0.99	0.28	0.83	0.82
Constant	-16.74	-16.75	-33.21	-10.64

Interactions factors (A: NaCl (%); B: pH; C: Heat; D: Cold; and E: Freezing time); C: Chloramphenicol; GM: Gentamicin; CRO: Ceftriaxone; and ST: Streptomycin; NI: not involved in the model; * shows a significant term. + and – indicate the increasing and decreasing effects of the terms on the antibiotic resistance.

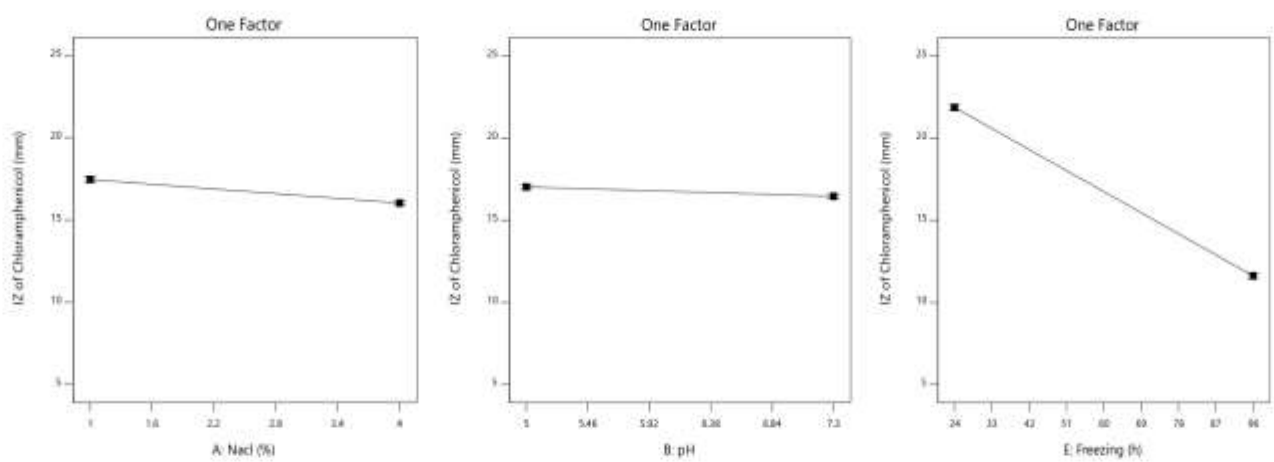

Figure 1. The main effects of the stress factors on the chloramphenicol resistance of S. Typhi. IZ: Inhibition Zone.

Exposure to food related stresses

Stress was applied simultaneously to the bacterial cocktails. For this purpose, 1 mL of fresh culture was added to TSB tubes prepared based on osmotic pressure (1% and 4% NaCl, Merck, Darmstadt, Germany) and acid (pH 5 and pH 7.3) stresses. Pure lactic acid (Merck) was used to adjust the pH of the solution. The tubes were then exposed to heat (45 and 55 °C for 5 min), cold (4 and 8 °C for 24 h), and freezing (-20 °C for 24 and 96 h) stresses according to the experimental design. Finally, 100 µL from each tube was transferred to tubes containing 9.9 mL of TSB and incubated at 37 °C for 20 h. After incubation, these cultures were used for antibiotic resistance tests (Kaboudari et al., 2024 a).

Antimicrobial disc susceptibility

After exposing the bacterial cocktails to the stresses, changes in their antibiotic resistance were assessed using the disc diffusion method. For this purpose, four antibiotics (chloramphenicol (C), 30 µg; gentamycin (GM), 10 µg; ceftriaxone (CRO), 30 µg; and streptomycin (ST), 10 µg), which are commonly used against *Salmonella* serotypes, were selected (Kaboudari et al., 2024a). After exposure to the stresses, the bacterial cocktails were cultured on Mueller Hinton agar (QUELAB, Montréal, Québec, Canada) with a thickness of 4 mm, and the antibiotic discs (Padtan Teb, Tehran, Iran) were placed on the surface of agar. The plates were incubated at 37 °C for 24 h. The inhibition zone (IZ) diameter was measured using an automatic caliper (CLSI, 2020). The control group (not exposed to stress) was used in the experiments, and all experiments were performed in triplicate.

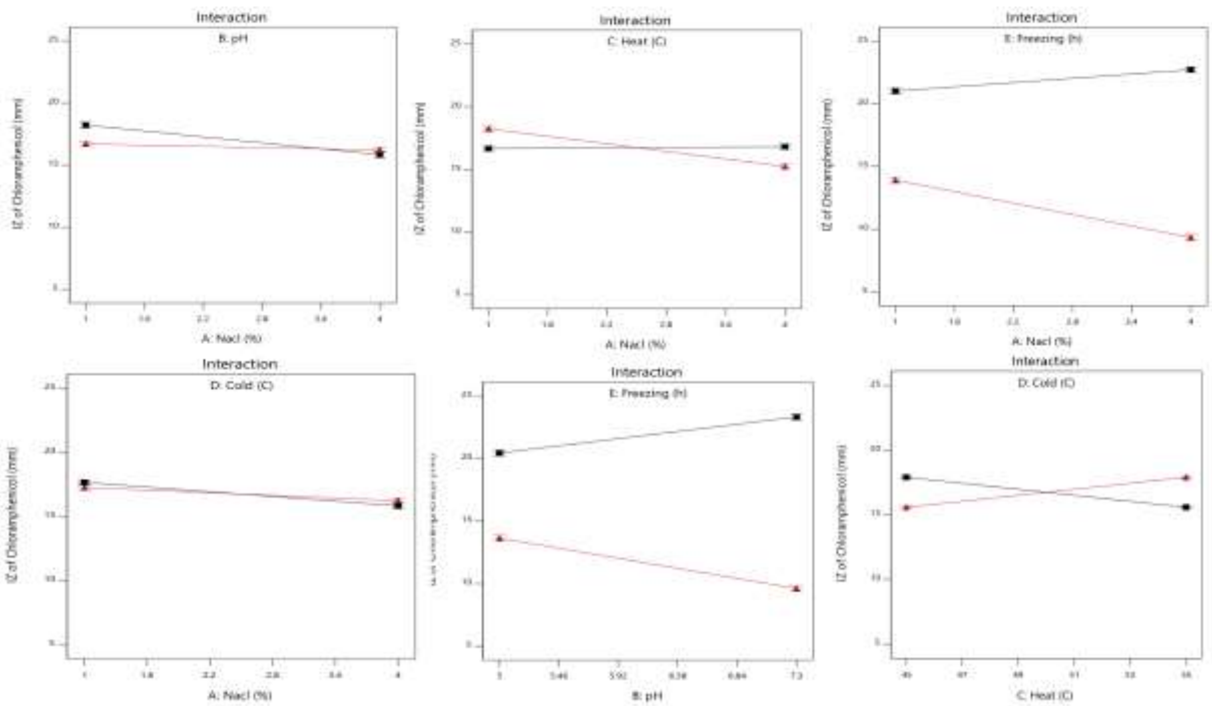

Figure 2. The interactive effects of the stress factors on the chloramphenicol resistance of *S.* Typhi. IZ: Inhibition Zone.

Experimental design and data analysis

The stress factors were osmotic pressure (**A**, NaCl percentage), acid (**B**, pH), heat (**C**, °C), cold (**D**, °C), and freezing time (**E**, h). Each factor was set at two levels. **Table 1** lists the factors and their levels. The responses measured were the IZ (mm) of each antibiotic against the three *Salmonella* serotypes. Design-Expert 12 software (Stat-Ease Inc., Minneapolis, USA) was used to design the experiment and analyze the data. The interactions between different stress factors were significant in this study. Therefore, a regular two-level factorial design with resolution V and a configuration of 25-1 was selected in the present work to estimate the main effects and interactions.

Results and Discussion

According to the results (**Table 2**), the most significant main effects on the antibiotic susceptibility of *S*. Typhi were freezing time (E) and NaCl concentration (A). Regarding the interactions of stresses, the interaction of pH with freezing time (BE), and NaCl concentration with freezing time (AE) were the most significant interactions affecting the antibiotic susceptibility of *S*. Typhi.

According to the results presented in **Figure 1**, increasing freezing time significantly increased chloramphenicol resistance (P<0.05). As shown in **Figure 2**, the interactions between NaCl and freezing, as well as pH and freezing stress, were the most significant interaction effects on the chloramphenicol resistance of *S*. Typhi. At a high level of freezing time (96 h), the antibiotic resistance increased with increasing NaCl concentration. However, a reverse pattern was observed at a low freezing time (24 h). A similar trend was observed for the interaction between pH and freezing stress. According to **Figure 3**, gentamicin resistance of S. Typhi was increased with decreasing heat temperature from 55 to 45 °C.

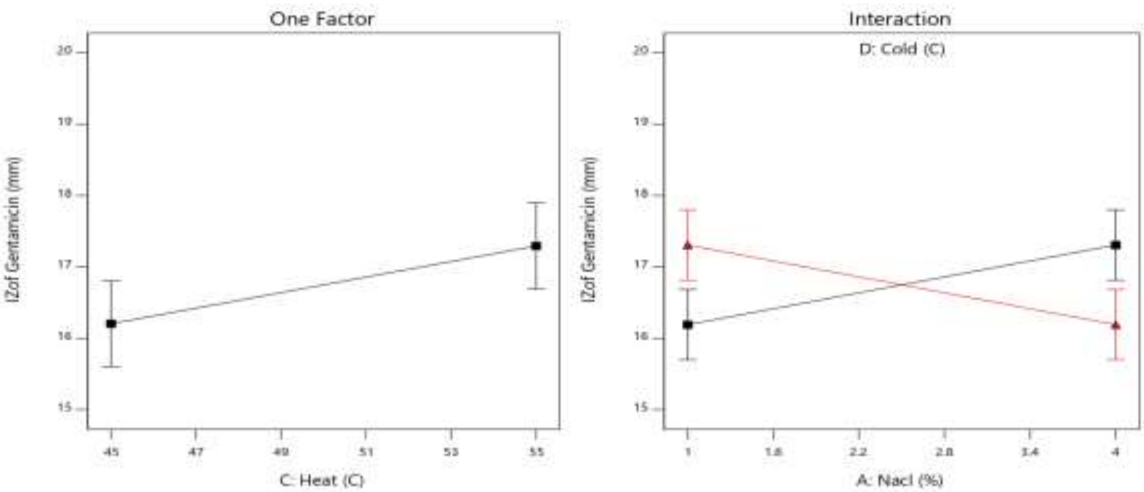

Figure 3. The main and interactive effects of the stress factors on the gentamicin resistance of *S.* Typhi. IZ: Inhibition Zone.

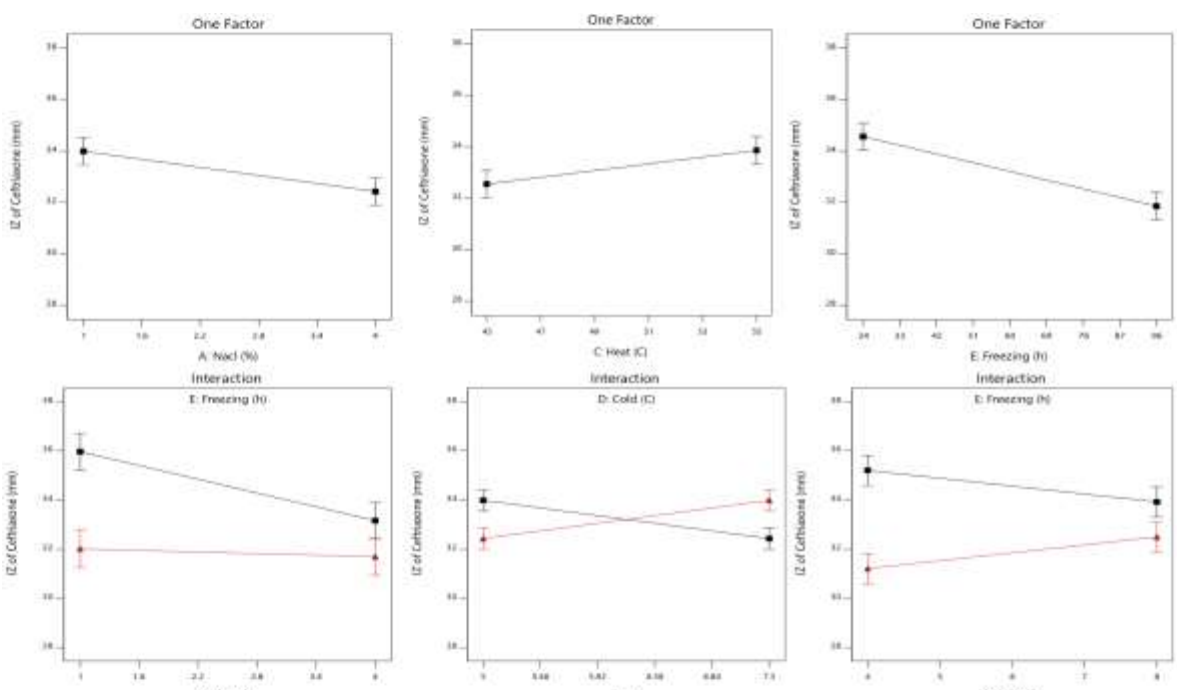

Figure 4. The main and interactive effects of the stress factors on the ceftriaxone resistance of *S.* Typhi. IZ: Inhibition Zone.

Resistance to ceftriaxone was significantly increased with increasing NaCl concentration and freezing time (**Fig. 4**). The most significant main effect was freezing time, which strongly increased ceftriaxone resistance in *S*. Typhi. Based on the results, the most significant interaction was found between pH and cold stress (**Fig. 4** and **Table 2**). **Figure 5** shows that resistance to streptomycin was decreased with increasing heating temperature. The most significant interaction was found between NaCl concentration and freezing time (**Fig. 5** and **Table 2**). At a low level of freezing time (24 h), resistance to streptomycin was decreased with increasing NaCl concentration. However, a reverse pattern was observed at a high freezing time (96 h). This study aimed to investigate the changes in antibiotic susceptibility/resistance of *S.* Typhi isolates under food-related environmental stress conditions. The results showed that the *S.* Typhi cocktail caused changes in antibiotic susceptibility in response to the applied stresses (either alone or in combination). All stress factors were effective, but freezing, osmotic pressure and pH had greater effects than the others. Several studies have been conducted on the effects of various stresses and shocks, such as osmotic pressure, non-lethal temperatures, and the presence of various substances, such as enzymes, on the growth, phenotypic, and genotypic characteristics of bacteria (Kaboudari et al., 2024 a,b; Liu et al., 2020). In this study, we investigated the effects of food-related stresses in single and combined forms on changes in antibiotic resistance in *S*. Typhi. Among the stressors studied, our results showed that the presence of stresses in a combined form (similar to the food model) can show more significant differences than the single factors.

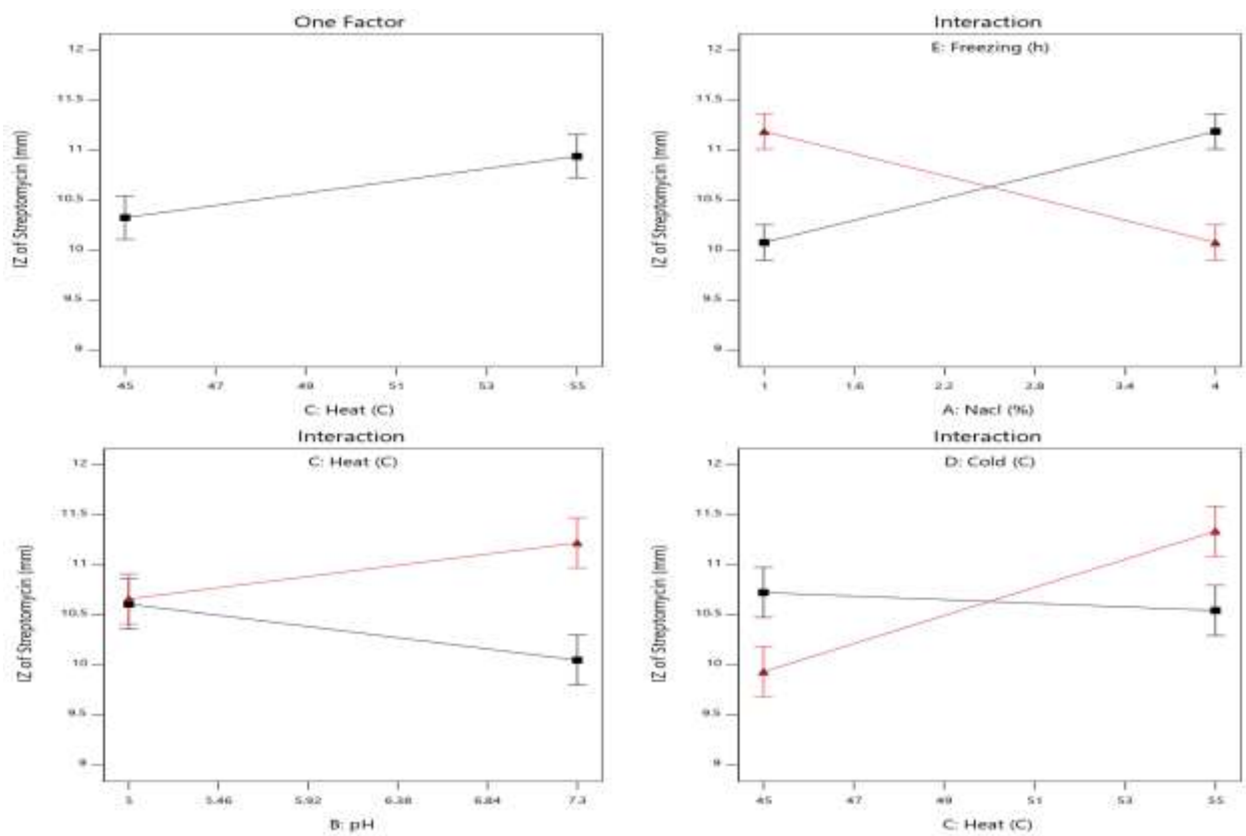

Figure 5. The main and interactive effects of the stress factors on the streptomycin resistance of *S.* Typhi. IZ: Inhibition Zone.

There are increasing reports of antibiotic resistance in food-borne *Salmonella* (Oh et al., 2025; Punchihewage-Don et al., 2024). A study from northern India showed that their resistance patterns to commonly used therapeutic antibiotics were nalidixic acid (66.7%), chloramphenicol (37%), co-trimoxazole (34.6%), cefotaxime (48.1%), and ciprofloxacin (18.5%) (Taneja et al., 2013). McMahon et al. investigated the effect of different concentrations of salt (NaCl) on the antibiotic resistance of *Salmonella* Typhimurium, *Escherichia coli*, and *Staphylococcus aureus*. Their results showed that salt concentrations above 4.5% increased the antibiotic resistance of the three bacteria (McMahon et al., 2007). Kang et al. investigated the thermal resistance of *Salmonella* Enteritidis to acidic, saline, and temperature stresses. The results of their study indicated that single-factor stress (acid or salt) increased the thermal resistance of bacteria (Kang et al., 2018).

Salmonella, one of the leading causes of foodborne illness worldwide, is often transmitted through animal products (Manafi et al., 2020). Antibiotic treatment is particularly important for immunocompromised individuals, such as older adults and immunocompromised patients, because of their increased susceptibility to severe effects. Multi drug resistance (MDR) *Salmonella*, which can develop after antibiotic use in food animals, may be transmitted to humans and lead to significant health challenges. *Salmonella* neutralizes antibiotics through mechanisms such as horizontal gene transfer via plasmids, regulation of the entry/exit system, and production of enzymes that inactivate or modify antibiotics. The rise of megaplasmids in *Salmonella* is particularly concerning, as it may enable resistance to a broader range of antibiotics (Oh et al., 2025). Recently, *S.* Typhi infection has been recognized as one of the most critical causes of gallbladder and pancreatic disease. Chronic *S.* Typhi infection plays a crucial role in the development of gallbladder carcinoma. One of the most important reasons for the involvement of *S.* Typhi in chronic disease is its ability of this foodborne pathogen to form biofilms. Antibiotic resistance and increased survival can ultimately lead to an increased ability to form biofilms (Nagaraja & Eslick, 2014; Karavolos et al., 2011). In a recent study by Pervaiz et al. (2025), *S.* Typhi and *E. coli* were isolated from food samples and examined. Beta-lactamase resistance genes were isolated from both bacterial species.

Conclusion

Given the importance of antibiotic resistance in public health and food safety, it is fundamental to investigate the processes leading to changes in the antibiotic resistance patterns of food bacterial serotypes. To develop methods to control and reduce antibiotic resistance, it is important to study and investigate phenotypic behavioral patterns. The results showed that various stressors that may be present in food during processing and storage can change (increase or decrease) the antibiotic resistance of food-borne *S.* Typhi. Food-related stresses include those that affect food microorganisms during food processing and storage can cause increasing the antibiotic resistance. Food microorganisms are often simultaneously exposed to multiple stressors. These simultaneously stresses in food matrix can increase the antibiotic resistance in all stages of food productions; from farm to work.

Acknowledgments

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Conflicts of interest

None.

Disclaimer

None.

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