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Prevalence and Survival of *Salmonella* spp. in Raw Chicken: Risks in Online Delivery and Storage Systems

(Prevalens dan Kemandirian *Salmonella* spp. pada Ayam Mentah: Risiko dalam Sistem Penghantaran atas Talian dan Sistem Penyimpanan)

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ABSTRACT

Nontyphoidal *Salmonella* is one of the most significant foodborne pathogens found mainly in poultry, with *Salmonella enterica* subp. *enterica* serovar Typhimurium is the most common cause of poultry-related outbreaks. This study aimed to determine the prevalence of *Salmonella* spp. and *S*. Typhimurium in raw chicken purchased from online stores and to evaluate the shelf-life of raw chicken stored in frozen (-18 °C) and chilled (4 °C) conditions. A total of twenty-seven (n=27) samples were purchased in three consecutive months. Detection of *Salmonella* spp. and *S*. Typhimurium were determined using standard ISO 6579-1:2017 and molecular methods. Results show that all brands purchased online were contaminated with *Salmonella* spp. and *S*. Typhimurium, with Brand B exhibiting the highest prevalence of *Salmonella* spp. (100%) and Brand A, showing the highest prevalence of *S*. Typhimurium (66.7%). Chicken wings showed significantly higher contamination ($p < 0.05$) compared to breast and thigh parts. Shelf-life analysis combined with predictive modelling showed that freezing is effective in preserving raw chicken, with a daily inactivation rate of Total Viable Count (TVC) (0.23%) and *Salmonella* spp. (0.45%), which is also predicted to be safely stored up to 9 months in a freezer. Under chilled storage, the raw chicken acceptance limit was 0.38 days (≤ log 5 cfu/g), while the spoilage threshold was 4.2 days (≥ log 7 cfu/g). The results of this study suggest that raw chicken purchased from online stores is potentially contaminated with *Salmonella* spp. thus, safe handling and delivery need to be practised to reduce the risk.

Keywords: Online stores; prevalence; *Salmonella* spp.; shelf-life; TVC

ABSTRAK

Salmonella bukan tifoid merupakan salah satu patogen bawaan makanan yang utama dan kebanyakannya dari ayam dengan *Salmonella enterica* subp. *enterica* serovar Typhimurium adalah yang paling kerap dikaitkan dengan wabak penyakit berkaitan ayam. Kajian ini bertujuan untuk menentukan prevalens *Salmonella* spp. dan *S*. Typhimurium pada ayam mentah yang dibeli secara atas talian dan menilai ujian jangka hayat ayam mentah yang disimpan pada suhu sejuk beku (-18 °C) dan sejuk (4 °C). Sebanyak dua puluh tujuh sampel (n=27) dibeli daripada tiga jenama yang berbeza dalam tempoh 3 bulan. Pengesanan *Salmonella* spp. dan *S*. Typhimurium dilakukan menggunakan piawai ISO 6579-1:2017 dan kaedah molekular. Keputusan menunjukkan bahawa semua jenama tercemar dengan *Salmonella* spp. dan *S*. Typhimurium, dengan Jenama B menunjukkan prevalens tertinggi bagi *Salmonella* spp. (100%) dan Jenama A bagi *S*. Typhimurium (66.7%). Kadar pengasingan lebih tinggi secara signifikan ($p < 0.05$) pada bahagian kepak berbanding dada dan peha. Keputusan ujian jangka hayat dan model ramalan menunjukkan bahawa suhu sejuk beku mampu menghalang pertumbuhan bakteria dengan kadar penyahaktifan sebanyak 0.23% bagi jumlah bilangan boleh hidup (TVC) dan 0.45% bagi *Salmonella* spp. serta menunjukkan jangka hayat ayam mentah selamat disimpan sehingga 9 bulan. Selain itu, model ramalan juga telah menentukan tahap penerimaan ayam mentah pada 0.38 hari (≤ log 5 cfu/g) dan tahap kerosakan pada hari ke 4.2 (≥ log 7 cfu/g) pada penyimpanan suhu sejuk. Kajian ini mendapati bahawa ayam mentah yang dibeli secara atas talian berpotensi dicemari dengan *Salmonella* spp., oleh itu protokol penyimpanan dan penghantaran ayam mentah yang betul perlu dipraktikkan.

Kata kunci: Kedai atas talian; prevalens; *Salmonella* spp.; TVC; ujian jangka hayat

INTRODUCTION

Poultry products are a major cause of salmonellosis in developing countries such as Malaysia, India, Egypt, Brazil, and Zimbabwe. It is estimated that *Salmonella* causes 155,500 mortalities and 1.3 billion cases of salmonellosis annually (Sun et al. 2021; Yang et al. 2013). In 2018, *Salmonella* was responsible for 91,856 foodborne infections, making it the second most common cause of foodborne illness in the EU (European Food Safety Authority, European Centre for Disease Prevention and Control 2015). Detection of *Salmonella* in poultry products is often due to cross-contamination that occurs during production, processing, distribution, retail marketing, handling and preparation (Dookeran et al. 2012). In the majority of cases, *Salmonella enterica* subp. *enterica* serotype Typhimurium is the most prevalent pathogen. Consumption and handling of poultry and meat products at the retail level are frequently linked to foodborne illnesses globally. The microbial quality of poultry products is usually monitored by TVC levels and the presence of spoilage microorganisms. In general, a TVC below 10^7 cfu/g is considered acceptable for fresh poultry meat, while counts exceeding 10^7 cfu/g are typically associated with spoilage and contribute to product deterioration (Franke et al. 2017; Spyrelli et al. 2021). In addition to pathogenic microorganisms, particularly *Salmonella* spp., most countries have enforced a zero-tolerance policy for *Salmonella* in poultry products (O'Bryan, Ricke & Marcy 2022; WHO & FAO 2007).

Identification and isolation of *S*. Typhimurium are often found in the environment, especially in livestock farms. Animals may become contaminated with this pathogen if it is introduced from one farm to another through livestock trade or water used in the livestock process. Subsequently, this pathogen can spread in the food chain due to cross-contamination that occurs in slaughterhouses during the meat cutting process, the packaging process and contamination during handling and delivery. In Malaysia, a study conducted by Ibrahim et al. (2024) found that *S*. Typhimurium is one of the main serovars infecting poultry animals in the country. Analysis of data for ten years from 2011 to 2020 showed that out of 391 recorded cases, *S*. Typhimurium is among the eight most dominant serovars in the poultry industry. Therefore, raw chicken meat is one of the carriers of food-borne *Salmonella* (Abouzeed et al. 2000).

Time-temperature abuse has been identified as one of the most prevalent contributing factors to foodborne illness in general and salmonellosis outbreaks, with strong evidence linking it to improper food handling practices (European Centre for Disease Prevention and Control 2015; WHO 2008). In the food industry, storage temperature is a crucial control point, as *Salmonella* spp. can multiply at temperatures above 5 °C. Additionally, the research by Akbar and Anal (2015) analysed *S. enterica* behaviour, which was inoculated into poultry meat that was kept at 6 °C ± 2 °C for 35 days, the first seven days showed a 2.0 log CFU g^{-1} increase. Meanwhile, Dominguez and Schaffner (2009) suggest that *Salmonella* can persist on frozen processed chicken products for more than 16 weeks and on frozen beef trimmings for more than 9 months without significantly altering its growth. Food safety and shelf life are improved by controlling food temperature to keep it out of the danger zone by inhibiting microbial growth.

The globalization of food retail systems has affected the marketing and distribution of fresh food. Online grocery shopping has become increasingly popular among consumers as a result of the COVID-19 pandemic (Shen, Namdarpour & Lin 2022; Tyrväinen & Karjaluoto 2022). The increase in online grocery shopping has been driven by several factors that provide advantages to consumers. These factors are convenience, risk perception, reliability, value for money, and social influence (Tyrväinen & Karjaluoto 2022). However, online grocery shopping has drawbacks regarding raw material spoilage, as it necessitates stringent temperature and time control during delivery to consumers. Studies by Smadi et al. (2012) showed that *Salmonella* spp. can increase rapidly when raw chicken is exposed to unsafe refrigeration temperatures. In addition, improper handling during storage and transportation can lead to the risk of cross-contamination and will reduce the quality and freshness of the raw product.

Recently, most studies have only investigated *Salmonella* survival in chicken meat throughout the farm-to-retail chain; very few studies have assessed post-retail phases, such as delivery systems. Refrigeration (4 ºC) and freezing (-18 ºC) are standard temperatures used for storing chicken meat in retail stores and consumer households. However, temperature fluctuations and prolonged storage times are common, especially during online deliveries, and remain poorly understood. The first objective of this study was to identify and isolate *Salmonella* spp. and *S*. Typhimurium in raw chicken samples purchased through online delivery. The second objective was to assess the raw chicken shelf life over a 30-day period by quantifying the log cfu/g of the TVC and *Salmonella* spp. Next, predictive microbiology was conducted to evaluate the quality level of raw chicken meat. The findings of this study will provide valuable information about the microbiological safety of online food delivery, contributing to the development of safe delivery protocols for raw poultry.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Prevalence study samples Raw chicken samples were purchased online from online stores located in Selangor, Malaysia, for three consecutive months. A total of 27 samples from three different brands (A, B, C), consisting of three different chicken parts (thigh, breast, wing) were used in this study. The samples were ordered and transported to the laboratory on the same day under standard delivery protocols, with each sample kept in an insulated ice box to maintain a temperature of 4 ºC. Upon arrival, the samples were allowed to defrost at room temperature and were immediately used for analysis.

Shelf life study samples Raw chicken samples were purchased directly from a factory located in Selangor, Malaysia, to ensure the initial microbial consistency. All raw chicken that had been labelled was kept in the insulated ice box to prevent microbial growth during transit and divided into two groups: chiller (4 ºC) and freezer (-18 ºC) once it arrived in the laboratory for further analysis. The storage duration for the shelf-life study is 30 days. Samples in the chiller were stored for 1, 3, 6, 12, 18, 24, and 30 days, while samples in the freezer were stored for 6, 12, 18, 24, and 30 days.

DETERMINATION OF TOTAL VIABLE COUNT (TVC)

The TVC in raw chicken meat was determined using the spread plate technique on total plate count agar (TPC) (Oxoid, UK) (Beuchat et al. 1998). Approximately 25 g of the raw chicken meat was weighed and homogenously mixed with 225 mL of Buffer Peptone Water (BPW) using a stomacher to obtain a dilution of 10^{-1}. The resultant samples were then serially diluted tenfold in sterile distilled water to obtain dilutions ranging from 10^{-3} to 10^{-6}. Each dilution was pipetted 0.1 mL onto TPC in triplicate and incubated at 37 ºC for 24 h. The number of colonies on TPC was counted using the colony counter and expressed as colony forming units per millilitre (cfu/g) (Feng 2007).

DETECTION AND ISOLATION OF *salmonella* spp.

Detection and isolation of *Salmonella* spp. and *S*. Typhimurium were performed according to ISO 6579-1:2017 (Detection of *Salmonella* spp.) (Mooijman 2018). Selective agar used to isolate *Salmonella* spp. is MacConkey agar, Salmonella Shigella agar, Xylose-Lysine- Deoxychocolate agar (BD, Japan) and incubated at 37 °C for 24 to 48 h.

For the prevalence study, the identification of presumptive *Salmonella* spp. was further confirmed using biochemical tests and molecular identification methods. Primer sequences of ST 11 5' GCC AAC CAT TGC TAA ATT GGC GCA 3' and ST 15 5' GGT AGA AAT TCC CAG CGG GTA CTG G 3' specific to *Salmonella* spp., Fli15 5' AGC GGG TTT TCG GTG GTT GT 3' and Typ04 5' ACT GGT AAA GAT GGC T 3' specific to *S*. Typhimurium were used (Diana, Pui & Son 2012). A total volume of 25 µL was prepared containing 12.5 µL 2X Rapiq Taq Master Mix (Vanzyme, China), 5.5 µL distilled water, 1 µL of primer, and 5 µL DNA template. The thermocycler conditions were 2 min at 94 °C; 40 cycles of 45 s at 94 °C; 1 min at 53 °C, 1 min at 72 °C; and 7 min at 72 °C for the last cycle. 1.5% (w/v) agar was used for gel electrophoresis assay (Thung et al. 2018). In the shelf-life study, the raw chicken was enriched with Buffered Peptone Water (BPW) for 24 h and then enumerated on XLD agar (BD, Japan); the presumptive *Salmonella* colonies were expressed as (cfu/g).

DATA ANALYSIS AND PREDICTIVE MICROBIOLOGY MODELLING

Based on the experimental shelf life data, the primary models of Baranyi and Roberts (no lag) were fitted for TVC (chiller condition) using ComBase (USDA, USA), while the First Order Decay was fitted for TVC (freezer condition) and *Salmonella* spp. (chiller and freezer conditions) using JMP Statistical Software (SAS Institute, USA). The goodness of fit for both models was evaluated using standard error (SE), mean square error (MSE), and R-squared (R^2). All the data obtained were incorporated into the equations below to predict the microbiological safety of raw chicken products.
Baranyi and Roberts Equation:

$$N(t) = N_0 + \mu\max \,.\, t - \ln\left(1 + \frac{e^{\mu max.t} - 1}{e^{K-No}}\right)$$

(Baranyi & Roberts 1994)

where N(t) is the log cfu/g at time (t); N_0 is the initial value; µ is the maximum value; and K is the final value.
First- Order Decay Equation:

$$\log_{10} N(t) = \log_{10} N_0 - k.t$$ (Peleg 2006)

where N(t) is the log cfu/g at time (t); N_0 is the initial value; and *k* is the decay rate constant.

STATISTICAL ANALYSIS

A one-way ANOVA followed by Tukey's test was performed to determine significant differences ($p < 0.05$) in the isolation of *Salmonella* spp. and *S*. Typhimurium for three different brands and three different raw chicken parts. The populations of the TVC and *Salmonella* spp. obtained in different storage conditions were also evaluated for significant differences ($p < 0.05$) using one-way ANOVA followed by Tukey's test. Statistical analyses were performed using Minitab Statistical Software version 21 (Pennsylvania, US) (Silva & de Azevedo 2002).

RESULTS & DISCUSSION

PREVALENCE OF *Salmonella* spp. AND *S*. Typhimurium IN ONLINE PURCHASE RAW CHICKEN

The results of this prevalence study demonstrated that *Salmonella* spp. and *S*. Typhimurium were present in all the chickens purchased from three different brands of online stores (Table 1). Brands A and B had high *Salmonella* spp. prevalence, with 88.9% and 100%, respectively, while Brand C had a lower prevalence rate of 44.4%. The *S*. Typhimurium prevalence proportions were different for all brands. Brand A had the highest prevalence with 66.7%, while Brand B had 44.4%, and Brand C had 33.3%.

The findings show variation in the contamination of *Salmonella* spp. and *S*. Typhimurium across onlined-purchased chicken brands. This suggests that brand-specific processing and handling practices in factories play a critical role in pathogen prevalence. Previous studies have reported the detection of *Salmonella* spp. and *S*. Typhimurium at retail outlets such as, wet markets, supermarkets or butcher shops, which serve as major vendors of chicken meat in several countries. A study by Shafini et al. (2017) showed that the highest presence of *Salmonella* spp. in raw chicken was detected in wet market samples (35.4%), followed by supermarkets (26.9%) and butcher shops (21.3%). Besides, a study by Thung et al. (2016) also showed a high occurrence of *Salmonella* spp. (26.7%) and *S*. Typhimurium (3.3%) in chicken products taken from wet markets, compared to those from supermarkets. A similar result was obtained in this study, which also identified raw chicken as a reservoir for *Salmonella* spp. *Salmonella* spp. could present at various retail points where chicken is purchased, particularly from online purchases and wet markets. The key parameter for controlling contamination at these locations is the implementation of proper handling and hygiene protocols throughout the supply chains.

In comparison with all retail outlets available for purchasing raw chicken, online outlets seem to surpass the prevalence of *Salmonella* spp. and *S*. Typhimurium. This could be due to temperature abuse during the storage and online delivery process. While traditional retail outlets have fixed temperature storage and allow consumers to immediately refrigerate perishable items, the extended transit times and variable temperature conditions associated with online grocery delivery may create opportunities for *Salmonella* spp. and *S*. Typhimurium to multiply and grow. Morey and Singh (2012) conducted a study on the growth of *S*. Typhimurium in a chicken meat homogenate to simulate the natural environment of poultry meat at various temperatures above 4 ºC over six days. The results showed significant growth of *S*. Typhimurium, with an increase of 2 log cfu/g observed in the samples, confirming its ability to proliferate under these conditions. However, data on the prevalence of *Salmonella* spp. and *S*. Typhimurium purchased through online platforms is limited.

The results showed that chicken parts (wing, breast and thigh) significantly contribute to the presence of *Salmonella* spp. (P = 0.0002) and *S*. Typhimurium (P = 0.0135) as shown in Table 2. Brand A showed the highest *Salmonella* spp. and *S*. Typhimurium isolation, with no significant difference observed between the chicken parts.

TABLE 1. Prevalence of *Salmonella* spp. and *S*. Typhimurium on raw chicken samples purchased from three different online stores

Chicken parts	Number of positive sample					
	Brands A		Brand B		Brand C	
	Salmonella spp.	*S*. Typhimurium	*Salmonella* spp.	*S*. Typhimurium	*Salmonella* spp.	*S*. Typhimurium
Breast	3/3	2/3	3/3	0/3	1/3	0/3
Thigh	3/3	3/3	3/3	2/3	0/3	0/3
Wing	2/3	1/3	3/3	2/3	3/3	3/3
Prevalence (%)	8/9 (88.9)	6/9 (66.7)	9/9 (100.0)	4/9 (44.4)	4/9 (44.4)	3/9 (33.3)

TABLE 2. Isolation of *Salmonella* spp. and *S*. Typhimurium from three different chicken parts

Brand	Number of positive isolate (%)							
	Salmonella spp.				*S*. Typhimurium			
	Thigh	Breast	Wing	P value	Thigh	Breast	Wing	P value
A	34^{bc}	37^{abc}	18^{bc}	0.0002	20^{ab}	13^{ab}	5^{ab}	0.0135
B	33^{ab}	6^{bc}	22^{bc}		14^{ab}	0^{b}	5^{ab}	
C	3^{bc}	0^{c}	21^{a}		0^{b}	0^{b}	15^{a}	

[a-c] Different superscript letters indicate significantly different ($p < 0.05$) for *Salmonella* spp. and *S*. Typhimurium, respectively, based on Tukey's comparison test. P values denote overall significance across all brands and chicken parts

In contrast, Brand C had the lowest overall isolation rates, with the wings being the most contaminated part for *Salmonella* spp. and *S*. Typhimurium isolates. There is a high significant difference ($p < 0.05$) of the isolates in the wing compared to the breast and thigh in Brand C. In addition, the isolation of *Salmonella* spp. for wings of Brand C differed significantly ($p < 0.05$) from those in Brands A and B. However, most of the studies showed the opposite trend, that *Salmonella* spp. and *S*. Typhimurium found in raw chicken are more highly contaminated in breasts compared to wings (Oscar 2014; Thung et al. 2016).

The variation of contamination levels across different chicken parts may be influenced by factors such as hygiene protocols, equipment sanitation, and personnel handling during processing. These factors may also account for the higher contamination rate observed in the thigh of Brand B, where the isolation of *Salmonella* spp. and *S*. Typhimurium is more prevalent in the thigh than in the breast and wing. Moreover, regional location and hygienic practices of the slaughterhouse may also influence the observed results. Although less common, some studies have reported that a high prevalence of *Salmonella* spp. was found in turkey wing skin compared to drumstick and thigh skin (Peng et al. 2016). This implies that contamination can vary based on the type of poultry and the practices that occur in the slaughterhouse and during packaging.

SHELF – LIFE OF RAW CHICKEN STORED AT CHILLED AND FROZEN CONDITIONS

Proper storage of raw chicken is a primary factor in stopping the growth and multiplication of bacteria. Both sellers and consumers should store the chickens properly, especially when purchased through online delivery, as this study found that a large number of samples were contaminated with *Salmonella* spp. Raw chicken shelf-life stored in a chiller (4 ºC) and freezer (-18 ºC) showed significant differences in the microbial count of TVC in Figure 1(a). The growth of TVC observed in this study followed the normal microbial growth curve, with a significant increase ($p < 0.05$) from day 0 to day 3, which shows that the growth of microbes is in the log phase. In the following days, the TVC counts stabilized and showed no significant differences suggesting the microbial population in the stationary phase. However, at this stage, microbial loads are considered unsafe for human consumption. It is in line with Dourou et al. (2021), which reported a significant increase ($p < 0.05$) in TVC count in chicken breast and thigh fillet from day 0 to day 5 stored at 4 ºC ± 1 °C. In addition, their study also show that psychrotrophic bacteria such as *Pseudomonas* spp., *B. thermosphacta* and lactic acid bacteria (LAB) dominate the microbial population under chilled storage. These bacteria are capable of surviving at low temperatures and responsible for the initial increase in TVC, followed by stabilization during the stationary phase.

Meanwhile, frozen storage at -20 ºC for the growth of TVC showed a lower significant difference ($p < 0.05$) from day 0 to day 12, after which the population remained stable from day 12 to day 30 without any significant changes. The initial decrease in the population can be attributed to the

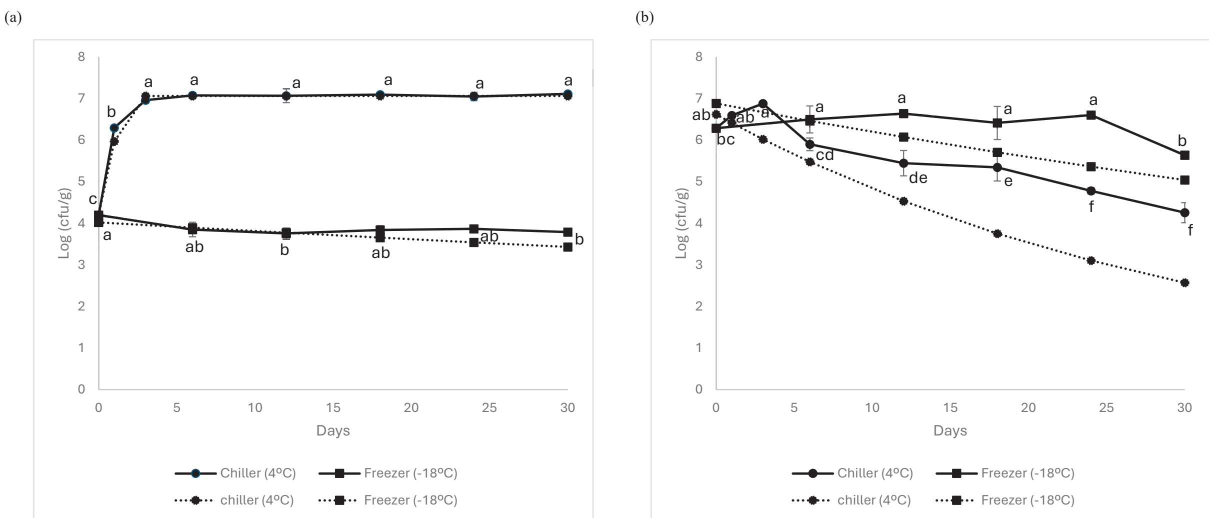

FIGURE 1. (a) Effect of storage at chiller (4 °C) and freezer (-18 °C) temperatures on microbial load (TVC) over a 30-day period. (b) Effect of chiller (4 °C) and freezer (-18 °C) storage on *Salmonella* spp. during the same period. Different alphabet letters (a-f) indicate significant differences ($p < 0.05$) based on Tukey's test for the experimental data. Solid lines (——) represent experimental results, while dotted lines (·········) represent values predicted by the applied predictive models

fact that only psychrotrophic bacteria survived in the raw chicken samples in frozen storage conditions. Oh and Lee (2024) reported that freezing at -20 °C does not eliminate psychrotrophic bacteria, which can resume growth when frozen food is thawed.

Figure 1(b) represents the shelf life of *Salmonella* spp. in raw chicken stored at a chiller (4 ºC) and freezer (-18 ºC) conditions. The growth of *Salmonella* spp. in chiller condition (4 ºC) increased and showed a significant difference ($p < 0.05$) from day 0 to day 3, followed by a significant decline ($p < 0.05$) from day 6 to day 30. The results align with Bruckner et al. (2012), who reported that spoilage *Pseudomonas* spp. are dominant and proliferate rapidly in fresh poultry, outcompeting *Salmonella* spp. and leading to a decrease in their population after the initial increase. This finding suggests that raw chicken is not suitable to store in refrigerated temperatures for extended periods, as it can lead to an increase in TVC and *Salmonella* spp. count as early as 1 day.

Meanwhile, the growth of *Salmonella* spp. under freezer conditions (-18 ºC) showed a significant decrease ($p < 0.05$) from day 6 to day 30. During the period between day 6 and day 24, fluctuations in *Salmonella* spp. counts were observed, but these changes were not statistically significant ($p > 0.05$). By day 30, a significant decrease in *Salmonella* spp. was observed, indicating a decline in bacterial survival over prolonged frozen storage. This variability may be attributed to differences in the initial *Salmonella* spp. load among the raw chicken samples, which could have affected the log CFU/g values and contributed to the observed fluctuations throughout the 30-day storage period. However, many studies have reported that the growth of *Salmonella* spp. remained constant during storage below 0 ºC for a long period (Bailey et al. 2000; Dominguez & Schaffner 2009). Additionally, some studies, such as Pradhan et al. (2012), have observed a decline in *Salmonella* spp. after prolonged frozen storage (21 days). This aligns with our result, which showed a significant decrease ($p < 0.05$) in *Salmonella* spp. from day 24 to day 30. *Salmonella* spp. cell may experience cumulative effects of cold shock and membrane damage that can cause a significant decrease over time in frozen storage conditions (Dominguez & Schaffner 2009). Thus, these results showed that the growth of TVC and *Salmonella* spp. completely halted during frozen storage, making freezing an effective method for long-term preservation.

PREDICTIVE MICROBIOLOGY

Model validation was performed using multiple goodness-of-fit indicators, including the coefficient of determination (R^2), standard error of fit (SE), and mean square error (MSE), to ensure accurate predictive validation (Tables 3 & 4). Visual validation is further demonstrated in Figure 1, which shows the alignment between experimental data and predicted values for *Salmonella* spp. and TVC throughout the entire storage period under chiller and freezer conditions. In a chiller condition (4 ºC), the growth of TVC fitted by the Baranyi and Roberts Model exhibited an excellent fit ($R^2 = 0.997$, SE = 0.054) compared to the First Order Decay ($R^2 = - 6.660 \times 10^{-16}$). This indicates TVC undergoes a growth phase under chiller conditions rather than microbial inactivation. As shown in Figure 1(a), the model demonstrated consistent accuracy throughout the entire 30-day storage period, with predicted values closely aligning with experimental data at all time points. These results agree with previous studies that have successfully used the Baranyi and Roberts model to predict the growth of TVC under various conditions (Feng 2022; Feng, Drummond & Sun 2014). However, the growth of *Salmonella* spp. under chiller conditions (4 ºC) showed a declining trend, thus fitting the First Order Decay model ($R^2 = 0.910$, MSE = 0.083) was preferable compared to the Baranyi and Roberts Model ($R^2 = 0.850$). Visual validation in Figure 1(b) indicates that the predicted values for *Salmonella* spp. showed optimal alignment during the early storage phase (0-6 days). However, increasing deviation was observed toward day 30. Despite this, the overall trend in the predictive data remained consistent with the experimental observations throughout the storage period. These deviations may be caused by various biological factors that influence bacterial survival during the storage

TABLE 3. Comparison of the fitted (R^2 values) predictive model for microbial survival under chiller and freezer storage conditions

Storage Conditions	Microbial Analysis	R^2 values	
		Baranyi & Roberts Model	First Order Decay
Chiller (4 ºC)	TVC	0.997	-6.660×10^{-16}
Chiller (4 ºC)	*Salmonella* spp.	0.850	0.910
Freezer (-18 ºC)	TVC	N/A	0.417
Freezer (-18 ºC)	*Salmonella* Spp.	N/A	0.436

R^2; R-Squared, TVC - Total Viable Count, N/A – Not Available period. The findings are supported by Kinsella et al. (2006), who noted a significant reduction in *Salmonella* spp. cell viability under low temperature (4 ºC). The decrease in viable cells was linked to injury caused by initial osmotic stress and further damage during prolonged storage. Such factors can result in non-linear inactivation behaviour that is not fully matched by the First Order Decay model, which assumes a constant inactivation rate.

Under frozen conditions (-18 ºC), both TVC and *Salmonella* spp. were fitted only during the stable phase (after day 0) in the predictive model, reflecting the actual microbial behaviour in freezer conditions. The First Order Decay displayed a moderate fit for TVC ($R^2 = 0.417$, MSE = 0.019) and *Salmonella* spp. ($R^2 = 0.436$, MSE = 0.128), demonstrated that the microbial inactivation phases occur in freezer conditions. However, as shown in Figure 1(a) and 1(b), the model shows significant limitations, especially in predicting the shelf life of *Salmonella* spp. over extended periods. The models maintained good alignment up to approximately 12 days, after which predictions increasingly deviated from experimental values. This deviation suggests that a simple first-order kinetics model may not accurately represent the complex stability dynamics during extended frozen storage, highlighting a significant limitation of the current modelling approach. In frozen storage conditions, several factors may contribute to variation in microbial inactivation rates over time. These include cellular responses to cold-induced stress, changes in storage temperatures, protective components within the food matrix, and the occurrence of sublethal cell injury (Diez-Gonzalez 2019). TVC and *Salmonella* spp. may either become inactivated or regain viability depending on factors such as freezing intensity and potential temperature fluctuations. This variability in survival introduces non-linear dynamics in microbial growth patterns that are not adequately reflected by First-Order Decay models. This finding was supported by Bevilacqua et al. (2015), who reported that simple first-order kinetics often fail to accurately fit microbial data in complex environments or for populations that display a non-linear trend.

The predictive models in this study showed better accuracy for TVC than for *Salmonella* spp. This is likely because TVC includes a mix of spoilage bacteria that generally show steady growth or decline during storage. In contrast, *Salmonella* spp. is a pathogen that reacts more sensitively to stress, especially at low temperatures, resulting in an irregular survival pattern that makes it difficult to compare with predictive models. Similar observations were reported by Farakos, Schaffner and Frank (2014), who found that *Salmonella* survival under low water activity conditions was highly variable and did not always follow first order inactivation kinetics.

Incorporating the kinetic growth parameter into the Baranyi and Roberts equation provides valuable insight into the acceptance level and spoilage threshold of TVC in raw chicken (Table 4). Specifically, the model estimated that raw chicken stored at 4 ºC remains acceptable for up to 0.38 days (≤ 5 log cfu/g) and reaches complete spoilage at 4.2 days (≥ 7 log cfu/g) (Katiyo et al. 2020; Nørrung & Buncic 2008). The predictions using the Baranyi and Roberts Model (no lag) are consistent with shelf-life experimental data, where the TVC exceeded 5 log cfu/g by day 1 and 7 log cfu/g by day 6, suggesting the importance of proper storage conditions. This finding is consistent with the study by Katiyo et al. (2020), which reported significant physicochemical deterioration in raw chicken after day 7, when the TVC count exceeded 8 log cfu/g. Additionally, the log cfu/g of *Salmonella* spp. predicted by the First Order Decay model based on the TVC acceptance (t =0.38) and spoilage (t = 4.2) thresholds, remained high (> 6 log cfu/g) throughout the storage period. The high level of *Salmonella* spp. detected in both experimental and predicted results is attributed to the initial presence of detectable amounts and its ability to proliferate under chiller conditions (4 ºC). Notably, most food safety standards necessitate the absence of *Salmonella* spp. in 25 g of the product (Smajhel & Shadrova 2018). Although the TVC value of 4.94 log cfu/g remains within the microbiological acceptance limit (≤ 5 log cfu/g), these findings suggest that the product may still pose a potential food safety risk due to the possible presence of pathogenic bacteria such as *Salmonella* spp. Hence, raw chicken must be appropriately handled after storage in a chiller to ensure it is safe for consumption. The accuracy of estimating shelf-life data of raw chicken under chilled conditions using the microbial growth model is supported by the strong correlation between experimental data and predictive modelling.

This First Order Decay model of *Salmonella* spp. in chiller condition (4 ºC) also estimated a decay constant (k) of 0.0137, indicating a bacterial inactivation rate of 1.37% per day, which represents a moderate inactivation rate. In contrast, under freezer conditions (-18 ºC), the model estimates the inactivation rate of TVC (k = 0.0023 day^{-1}, 0.23% daily) and *Salmonella* spp. (k = 0.0045 day^{-1}, 0.45% daily), provides valuable insights into the effectiveness of freezing as a preservation method for raw chicken. A freezer stability test was conducted at t=270 days, based on the U.S Food and Drug Administration (FDA)'s recommended maximum storage duration for poultry (FDA 2018). According to the First Order Decay model, a slight reduction in TVC (0.625 log cfu/g) and a moderate reduction in *Salmonella* spp. (1.219 log cfu/g) were estimated over the 9 months storage period. These findings demonstrated that freezing significantly decreases growth rates by removing liquid water through ice formation and inhibits microbial cellular functions (Smith 2011). Previous studies have also confirmed that freezing is one of the most effective methods for preserving raw chicken (Al-Jasser 2012; Fernandes et al. 2016; Sabikun et al. 2019). However, estimating the constant decay rate under freezer conditions may not accurately represent the actual inactivation rate, as the reduction of TVC and

TABLE 4. Estimated kinetic parameters of TVC and *Salmonella* spp. from the fitted predictive model

Storage condition	Microbial analysis	Predictive Model	Microbial kinetic parameters (growth and decay)			Goodness of fit		*Quality Threshold			
			N_0 (log cfu/g)	μ_{max} (log cfu/g)	K (log cfu/g)	SE of Fit	R^2	Freshness		Spoilage	
								n(t) (log cfu/g)	t (days)	n(t) (log cfu/g)	t (days)
Chiller (4 ºC)	TVC	Baranyi and Roberts Model (no lag)	4.201 ±0.054	2.121±0.080	7.0657±0.022	0.054	0.997	4.94	0.38	7.07	4.2
			N_0 (log cfu/g)		*K* (day^{-1})	MSE	R^2	n(t)	t (days)	n(t)	t (days)
Chiller (4 ºC)	*Salmonella* spp.	First Order Decay	6.625 ± 0.161		0.0137±0.002	0.083	0.910	6.62	0.38	6.56	4.2
								Freezer Stability Test (t=270) (log cfu/g)			
Freezer (-18 ºC)	TVC	First Order Decay	4.025 ±0.010		0.0023±0.001	0.019	0.417	3.40			
Freezer (-18 ºC)	*Salmonella* spp.		6.889 ± 0.395		0.0045±0.003	0.128	0.436	5.67			

TVC; Total Viable Count, N_0; Initial Value, μ_{max}; Maximum Rate; K; Final Value; *K*; Rate Constant, SE; Standard error, MSE; Mean Square Error, R^2; R-Square,*Quality Threshold: (TVC), Freshness and spoilage levels at 4 ºC were calculated using the Baranyi and Roberts model (no lag) with the kinetic growth parameters shown in the table; (*Salmonella spp.*) at 4 ºC was calculated using First Order Decay based on the TVC acceptance (t = 0.38) and spoilage (t = 4.2) level with the microbial kinetic decay parameter showed in the table; Freezer stability test (-18 ºC) for TVC and *Salmonella* spp. at 270 days was estimated by the First Order Decay model with parameters shown in the table

Salmonella spp. does not occur at a constant rate over time. Although the moderate R^2 values are 0.417 (TVC) and 0.436 (*Salmonella* spp.), indicating some deviation from the experimental data, the small MSE values 0.01857 (TVC) and 0.12824 (*Salmonella* spp.) confirm reasonable predictive accuracy. These findings suggest that, although the model provides useful approximations, further aspects should be investigated in future research to enhance the accuracy of predictions regarding bacterial inactivation during chiller and frozen storage.

CONCLUSION

Raw chicken purchased from online stores showed a higher prevalence of *Salmonella* spp. and *S*. Typhimurium compared to other retail outlets. Therefore, control measures must be taken during the retail and after the post-retail phase by focusing on maintaining suitable storage conditions to prevent the growth of this pathogen. Retailers and consumers must be aware of the importance of preserving raw chicken at appropriate temperatures. Additionally, the government should educate the communities about safe practices when purchasing perishable products online.

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