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Survival Pattern of Enterohemorrhagic *Escherichia coli* Strain (CDC05) in Sterile Ground Beef Medium under different Physicochemical Conditions

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Abstract

The survival pattern of *Escherichia coli* O157:H7, an important foodborne pathogen of the enterohemorrhagic *E. coli* (EHEC) category, in different physicochemical conditions related to food storage, was evaluated in the study. The persistence of *E. coli* O157:H7 under conditions varying in salt concentration (0-12% w/v), pH levels (2-12), and storage temperatures encompassing ambient (28±2°C), refrigeration condition (4°C), and freezer condition (-18°C) conditions in sterile ground bovine meat medium was assessed. A mathematical model developed in the MATLAB analytical tool was used to study the survival kinetics of the pathogen across diverse stress conditions. It was observed that no viable cells were recovered after one week of freezing and two months of refrigeration. The shortest observed D-values for EHEC cells were 1.54 days at pH 12, 5.47 days at freezing temperature, and 26.72 days at refrigeration temperature in the sterile beef medium. Notably, *E. coli* O157:H7 exhibited prolonged viability exceeding thirty days under most stress conditions examined, indicating heightened resistance and persistence. The mathematical model demonstrated reliability in approximating survival durations and, thus served as a valuable predictive tool for risk assessment in various food processing steps, thereby ensuring effective management of foodborne pathogens.

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Introduction

Escherichia coli O157:H7 poses a significant public health challenge due to its ubiquitous occurrence and continuous implications in foodborne infections. Classified as an Enterohemorrhagic *E. coli* (EHEC) strain, this pathogen poses substantial risks to human health, causing gastrointestinal symptoms with serious sequelae like Hemorrhagic Colitis and Hemolytic Uremic Syndrome and often resulting in fatalities.[1] FAO/WHO[2] identified EHEC, including *E. coli* O157:H7, as a major microbiological risk in beef and related foods and emphasized effective risk assessment for the management of these pathogens. This pathogen has gained the attention of food safety scientists because of its high acquired virulence, extra-intestinal loci of infection, and alarmingly low infectious dose of 10-100 cells.[4]

Cattle are considered as the major reservoir of these pathogens and contaminated beef and bovine foods are the main source of human infections.[3,4,5] The ability of the EHEC strain, *E. coli* O157:H7, to persist and proliferate in food storage environments contributes significantly to its transmission and the incidence of foodborne diseases.[6]

The effective management of *E. coli* O157:H7 demands a thorough understanding of its survival patterns in different physico-chemical conditions that might encountered in food. Factors such as salt concentration, pH levels, and temperature are crucial in modulating the growth and, thereby the survival kinetics of this pathogen in food matrices. Predictions on survival and persistence of these pathogens in suspected food are key for devising effective strategies for the control of these pathogens in food. Predictive modelling is a valuable method for risk assessment of pathogenic microorganisms in foods.2 Mathematical modelling has been used for risk assessment and for predicting the growth, survival and death of microorganisms present in food.[2,7,8] Furthermore, developing a mathematical model based on experimental data will enable the extrapolation of survival kinetics, providing valuable insights into the behaviour of this pathotype of *E. coli* in complex environments in the food. Thus, the present study aims to systematically assess the survival patterns of *E. coli* O157:H7 in sterile bovine meat medium under diverse food storage-related conditions and to attempt a mathematical model for predicting the number of surviving cells under a fixed set of conditions.

Materials and Methods

Bacterial Strains

The survival study used the strain *E. coli* O157:H7, namely CDC05, isolated from a cow dung sample procured from Ernakulam, Kerala, India (9.980N. 76.260E). The bacterial isolate was identified and characterized by the methodology described by Joseph and Kalyanikutty[5] and Joseph *et al*.[9] The strain CDC05 was isolated by pre-enriching the cow dung sample in Novobiocin containing *Escherichia coli* (EC) broth, and enrichment using immunomagnetic bead (Dynabead®, Life Technologies, AB Systems, Norway), followed by a screening in Cefixime, Tellurite, and Sorbitol MacConkey agar medium.[5] The partial genetic characterization was done and can be assessed using the GenBank accession number, MT91268.[5] The optimal conditions for the growth of the strain were pH 7, salt concentration at 0.85% and 37°C.

Preparation of Bacterial Rnoculum

The bacterial cells were recovered from the glycerol stocks by inoculating them to Tryptone Soy Broth (TSB) (Himedia Pvt Ltd, Mumbai, India) and pure cultured by quadrant plating method in Tryptone Soy Agar (TSA). The inoculum for the stress survival experiments was prepared according to Elhadidy and Álvarez-Ordóñez[10,11] with slight modifications. For inoculum preparation, a single colony was transferred to 40 ml of TSB and incubated for 16 hours at 37 °C in a shaker incubator set to 160 rpm. Before inoculation, the OD610 value of the culture was adjusted to 0.02. The total number of viable cells in the inoculum was enumerated by the pour plate method to be around 10^8 cfu per ml. An aliquot of 100µl, which contains 10^7 robust cells, served as the initial inoculum to spike 200ml of the sterile ground beef medium before exposing to the corresponding physicochemical growth parameters.

Preparation of Food Medium

The survival pattern of the EHEC strain was assessed in the sterile minced beef medium. Boneless sliced raw beef was acquired from a retail meat shop and rinsed thoroughly in tap water. The meat was ground into a paste in a mixer grinder using sterile double-distilled water with a 1:1 (V/W)

proportion. Before autoclaving, an aliquot of 200ml of the minced beef was transferred aseptically to 500ml storage bottles (Borosil Ltd., Mumbai, India).

The minced meat medium for the temperature tolerance study was adjusted to pH 7 and with 0.85% sodium chloride level. The testing medium for the survival study in acidic or alkaline conditions was prepared by mixing minced meat with 200ml of pH-adjusted double-distilled water. The medium was adjusted to 0.85% sodium chloride. Similarly, the bottles of beef medium for testing osmotic stress tolerance were prepared by adding 200ml of double-distilled water pre-mixed with different sodium chloride levels ranging from 0-12%. After autoclaving, bottles were vigorously shaken to ensure the contents were evenly distributed.

Survival in different Temperature Conditions

The bottles were brought into respective storage temperatures before inoculation. All the experiments were done in duplicate and with an uninoculated negative control. Zeroth hour cell count was prepared by drawing samples just after the inoculation. Different temperatures selected for the survival study were room temperature (RT; 28+/-2^{o}C), refrigeration temperature (4^{o}C) and freezer temperature (-18^{o}C). The bottles were observed for three months.

At each sampling point, 1 ml of the thoroughly mixed sample was drawn and mixed with 9 ml of normal saline. Serial dilutions were prepared from this, and the surviving cells were enumerated by spread plate culture using TSA agar. Total survived cells were determined by multiplying the average of observed colonies on duplicate plates by the respective dilution factor.

Survival in Acidic/Alkaline Stress

Survival patterns of the CDC05 strain at different pH levels ranging from 3.5 to 12 were assessed. Before inoculation of strains, the pH of the sterile food medium was confirmed using pH testing paper strips (Himedia Pvt. Ltd., Mumbai, India). An aliquot of 1 ml was sampled at the intervals, namely 1st, 2nd, 3rd, 7th, 14th, 28th, and 60th, up to the 90th day, to enumerate surviving bacterial cells in the medium. The experiment was concluded when cell recovery on two consecutive samplings became zero.

Survival in Osmotic Stress Due to Different Sodium Chloride Concentrations

Osmotic stress to pathogenic bacterial cells was assessed at different sodium chloride concentrations (0%, 0.85%, 2%, 6%, 10% and 12%) in the beef medium. The pH of the bottles was adjusted to 7. Samples were collected at predetermined sampling intervals up to the 90th day of incubation to determine the total number of survived cells. All the tests were duplicated, and the average of observed colonies was used to calculate the population of survivors.

Data Analysis

The mean values of the survivors were given as mean ± SD. Common logarithms of the survived bacterial population at various storage conditions were plotted against the time intervals.

The data on the cell numbers that survived at different experimental conditions were used to prepare a set of theoretical values with the help of Eq. 1 given below: The data analysis was done in MATLAB (version 9.10, MathWorks, Natick, USA).

$$N = [N_0+N_{02}\,(1-P/7) +N_{04}\,(1-S/0.85) +N_{06}\,(1-T/301)] *\exp\{-((t-b)/c)^2\} \quad ...(1)$$

where N is the number of cells at any given time; N_{02}, N_{04}, and N_{06} represent the initial number of cells exposed to pH stress, salt stress and temperature stress, respectively; $[N_0+N_{02}(1-P/7)+N_{04}(1-S/0.85)+N_{06}(1-T/301)]*\exp\{-b^2/c^2\}$ is the initial number of cells inoculated at any condition; P represents the value of pH; S represents the value of sodium chloride concentration; T represents the value of temperature in Kelvin scale; The equation provided a reasonable fit to the data of all the experiments with different stress conditions. The data prepared from the theoretical Eq. 1 is used to plot the survival population against time in the graphs.

Regression analysis was performed to calculate the values of correlation coefficients and F-statistics using the Microsoft Excel tool.

Decimal-reduction time, also known as D-value, was determined following the methodology described by Elhadidy and Álvarez-Ordóñez.[10] The D-value is the time needed for one logarithmic reduction

of a bacterial population under specific storage conditions. The D-value was calculated using the Eq. 1:

$$D = b + \sqrt{(b^2+2.303*c^2)} \quad ...(2)$$

Where D is the decimal reduction time (min); b and c are constants. The sum of the constants, b + c, is the time required to reduce the population at a specified condition to 1/e or 63.2% of its initial number (N_0) when the other two conditions are kept at the optimal level.

The study examined the combined effects of pH stress, salt stress, and temperature stress on the variation in the total number of cells over time, focusing on the optimum pH of 7, salinity of 0.85% salt, and a temperature of 301 K, using Equation 1. The change in the cell numbers were found to be in an exponential manner. By keeping any two parameters constant at the optimum value, Eq. 1 enables the assessment of kinetics of cell reduction for different non-optimum values of the third parameter.

Results

Survival of EHEC Strain at different Stress Conditions

The persistence of the EHEC strain, CDC05, in various stress conditions related to the beef medium was assessed for about a three-month period, and the survival pattern in different storage temperatures, pH levels and salt concentrations is depicted in Figure 1.

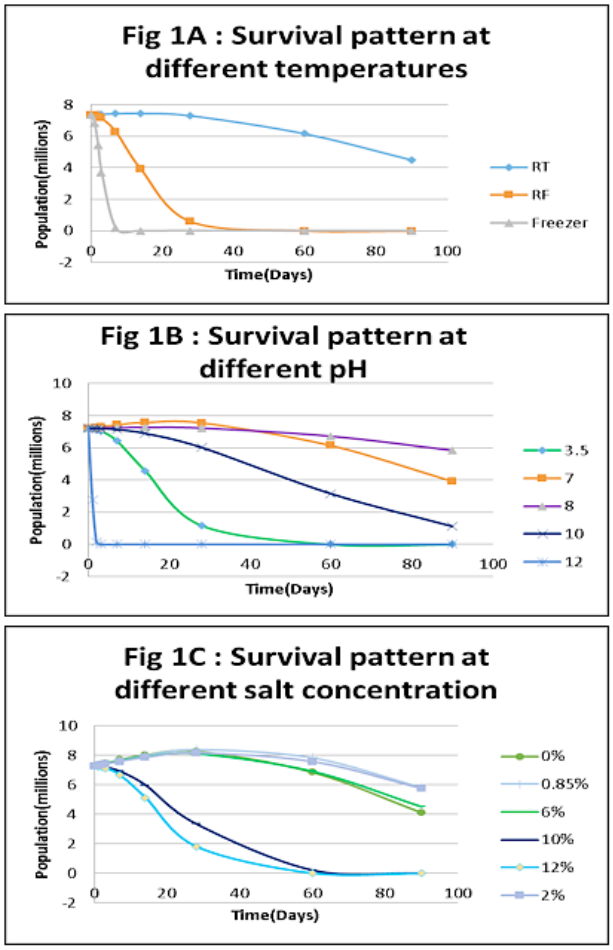

Fig.1: Survival pattern of Enterohemorrahagic *E. coli* strain, CDC05, in Ground bovine medium under different physicochemical conditions

Survival in different Storage Temperatures

The survival pattern of EHEC strain at ambient, refrigeration, and freezing temperatures is presented in Fig 1A. The logarithmic value of the population derived from the mathematical equation 1 is plotted against various stress conditions in the graph. Due to the predictive nature of Eq. 1, the survival rate at any given set of storage temperatures can be prepared.

Table 1: Comparison of theoretical and experimental values of CDC05 cells that survived under different conditions

Days	Survival in different salt concentrations (cell count in log10 values)											
	0%		0.85%		2%		6%		10%		12%	
	TV	EV	TV	EV	TV	EV	TV	EV	TV	EV	TV	EV
0	7.24	7.24	7.24	7.24	7.24	7.24	7.24	7.24	7.24	7.24	7.24	7.24
1	7.30	7.35	7.30	7.20	7.29	7.79	7.29	7.27	7.23	6.69	7.22	6.56
2	7.37	7.46	7.36	7.38	7.34	7.35	7.35	7.29	7.21	6.63	7.18	5.65
3	7.44	7.94	7.42	7.85	7.39	7.90	7.40	8.23	7.17	5.30	7.12	5.17
7	7.68	7.91	7.65	7.68	7.57	7.75	7.60	8.35	6.89	5.98	6.64	5.04
14	8.01	7.63	7.98	7.60	7.84	7.52	7.88	7.71	5.96	5.17	5.12	4.07
28	8.27	7.57	8.39	7.49	8.14	7.50	8.09	6.65	3.33	3.30	1.81	1.39
60	6.83	6.02	7.83	6.65	7.55	6.10	6.79	5.88	0.20	0.00	0.01	0.00
90	4.09	4.12	5.83	5.98	5.75	5.90	4.49	4.30	0.00	0.00	0.00	0.00
r-value *	0.938		0.696		0.926		0.954		0.983		0.977	
P-value**	0.001		0.034§		0.000		0.000		0.000		0.000	

Days	Survival in different temperatures (cell count in log10 values)					
	RT		FT		RF	
	TV	EV	TV	EV	TV	EV
0	7.34	7.34	7.34	7.34	7.34	7.34
1	7.35	7.76	6.83	4.19	7.31	4.94
2	7.36	7.87	5.43	3.62	7.25	4.06
3	7.37	7.98	3.70	3.42	7.13	4.02
7	7.41	7.91	0.16	2.17	6.27	3.99
14	7.43	6.61	0.00	0.00	3.90	3.69
28	7.28	5.77	0.00	0.00	0.58	3.14
60	6.15	5.20	0.00	0.00	0.00	0.00
90	4.50	4.47	0.00	0.00	0.00	0.00
r-value *	0.818		0.922		0.836	
P-value**	0.007		0.000		0.005	

Days	Survival in different pH (cell count in log10 values)									
	3.5		7		8		10		12	
	TV	EV	TV	EV	TV	EV	TV	TV	TV	EV
0	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20

1	7.18	7.18	7.23	7.50	7.20	6.82	7.19	6.66	2.75	3.11
2	7.13	7.56	7.27	7.80	7.21	7.10	7.19	7.10	0.14	0.00
3	7.05	7.63	7.31	7.73	7.22	7.41	7.18	7.09	0.00	0.00
7	6.43	5.78	7.43	7.86	7.24	7.74	7.11	5.99	0.00	0.00
14	4.58	4.53	7.57	7.06	7.26	7.62	6.88	5.27	0.00	0.00
28	1.17	3.84	7.55	6.91	7.22	7.49	6.00	5.08	0.00	0.00
60	0.00	0.00	6.14	6.57	6.72	7.00	3.13	3.38	0.00	0.00
90	0.00	0.00	3.93	4.00	5.85	5.80	1.10	1.07	0.00	0.00
r-value *	0.956		0.935		0.886		0.961		0.999	
P-value**	0.000		0.000		0.001		0.000		0.000	

Note: TV is theoretical value derived from Eq.1; EV is the experimental value; RT stands for room temperature; FT for freezer temperature and RF for refrigeration temperature: * r-value is the correlation coefficient value between EV and TV; ** P-value is the F-test statistic of significance between EV and TV. § The correlation is significant at 0.05% level.

The bacterial population in sterile ground beef was reduced from 2.17 x 10^6 cfu/ml to zero on the 7th day of the incubation period at -18°C. At refrigeration conditions (4°C), no cells were recovered on the 60th day of incubation. The initial population was 3.14 x 10^6 cfu/ml, and a steady decline in cells was observed after the first day. In the present study, the bacterial population in sterile ground beef remained considerably high (4.47 x 10^6 cfu/ml) even after three months of incubation at RT, which is 28+/-2°C. The observation points to the fact that the persistence of the pathogen in refrigerated and frozen food can lead to health and food safety issues. Owing to its high virulence and low infectious dose, the persistence of EHEC in foods is considered a significant public health threat.

Survival in Acidic or Alkaline Conditions

The strain was exposed to different scales of pH values (3.5, 7, 8, 10, 12) in the beef matrices at constant incubation temperature (RT) and salt concentration (0.85%). Survival patterns at different time intervals prepared using the mathematical Eq. 1 were presented in Fig. 1B. At pH 12, the 3.11 x 10^6 of the initial inoculum was observed to survive only for the first day. However, the bacterium tolerated pH 8 and 7, and the strain survived fairly well during the study period of 90 days.

The study demonstrated that the EHEC strain was tolerant to acidic conditions. At pH 3.5, only a two-log10 reduction in the number of cells was observed after 90 days of incubation. Among the stress conditions tested, pH 12 was found to be the most deleterious for the EHEC strains, as the death rate showed the highest value.

Survival of EHEC in Different Salt Concentrations

Similar to pH resistance, the EHEC strain showed good growth in low salt concentrations (Fig 1C). The salt concentrations of up to 6% have not affected the survival of bacterial cells in the beef medium. During the three-month experiment period, only a two-log decrease of cells was observed at salt concentrations of 2% and 6%. However, higher salt concentrations (10% and 12%) were detrimental to the cells, and no cell recovery was observed on the 60th day at 12% salt and the 90th day at 10% salt. The high salt concentration disrupts the osmotic balance of the cells and leads to their death.

The present study performed theoretical modelling with a set of survival data obtained from exposing the bacteria to a range of stress conditions, and the corresponding values are presented in Table 1. The strong agreement between theoretical and experimental values (p-value <0.01) substantiates the practical application of this computational model for predicting the survival of pathogenic cells in foods, especially in extreme food environments. The mathematical equation explained here used primary data from the experiments on the survival pattern of the EHEC pathogen in a sterile bovine meat medium under selected stress conditions for a period of 90 days. Using the given equation, the growth curve for any set of stress conditions can be regenerated. These survival models provide accurate predictions and

effectively manage processing set points to control the occurrence of pathogens in food matrices.

Thermal Death Time (D-value) at different Stress Conditions

Bacterial resistance to different stress conditions was calculated by determining D-values.[10] D-value is defined as the time in minutes required for the inactivation of 90% of the bacterial population. The greater the detrimental effect of the stress, the lower the D-value. The bacterium survived less than one week, with a minimum D-value at 12 pH and -20°C. Maximum D-value was observed for pH 7 and 8, salt concentration 0-6% and at room temperature. D-values calculated using the Equation are shown in Table 2.

Table 2: Thermal death time (D values) of EHEC strain under different stress conditions

Stress parameter	Value of b*	Value of c**	Thermal Death Time (D- value) in days
pH			
3.5	0.01	20.80	31.57
7	20.30	85.80	152.08
8	15.30	160.80	259.80
10	0.01	65.80	99.86
12	0.01	1.01	1.54
Salt Concentration (%)			
0%	27.30	74.80	144.05
0.85%	35.30	81.80	176.07
2%	33.33	95.80	182.45
6%	27.30	81.80	154.40
10%	0.00	31.80	48.26
12%	0.01	23.80	36.13
Temperature (^{0}C)			
Freezer	12.30	109.80	5.47
Refrigeration	0.04	17.58	26.72
Room	0.04	3.58	179.38

Note: *The D-value was calculated using the Equation, $D = b + \sqrt{(b^2+2.303*c^2)}$ where D is the decimal reduction time (min); b and c are constants related to the time required to reduce the population to 1/e of its initial number (N_0) at specified stress conditions.

Discussion

Several food safety failures that led to major foodborne outbreaks are due to improper food handling or temperature abuse during food storage and transportation.[12] The persistence of pathogens in food storage conditions can be a potent factor for spreading foodborne infectious diseases.[1] Foodborne pathogens endure a range of stresses within the food matrix and in food processing environments. These include temperature, oxygen availability, fluctuations of pH and osmolarity. Understanding these challenges is important for developing effective strategies to ensure food safety and protect public health.

In this study, different patterns of stress resistance in EHEC isolate were observed after exposure to multiple food-related stressors: temperature, pH and Salt concentration. Ground beef is considered an implicating agent in 33% of STEC-related outbreaks.[20] This was the reason for choosing the beef matrix for the survival analysis of this pathogen. At -18^{o}C the bacterial population in sterile ground beef survived till 7th day of the incubation period.

Strawn and Danyluk[13] demonstrated that the *E. coli* O157:H7 survived on mangoes and papayas when stored at a temperature of -20°C for a duration of a minimum of 180 days. The survival of cells in frozen storage is a concern as there is a potential risk of cross-contamination with other foods stored in the freezer. At refrigeration conditions (4°C), no cells were recovered on the 60th day of incubation. In raw milk, the *E. coli* O157 strain survives for over two months.[14] The organism was reported to survive in chocolates for more than one year[15] and in wheat flour for almost two years[16] at ambient temperatures. These reports also substantiate the psychrotrophic nature of the bacterium.

In the present study, the bacterial population in sterile ground beef remained considerably high even after three months of incubation at RT, which is 28+/-2°C. The observation points to the fact that the persistence of the pathogen in refrigerated and frozen food can lead to health and food safety issues. Owing to its high virulence and low infectious dose, the persistence of EHEC in foods is considered a significant public health threat.

Acidic stress is a stress that the organism may be exposed to in the environment, during food processing or in food matrices. In the present study, EHEC strain was tolerant to acidic conditions. In acidic foods like fruit juices with a low pH of 3.5, the persistence of EHEC strain might pose safety concerns.[16] However, EHEC bacteria are known for their stress tolerance at various pH conditions, ranging from highly acidic to alkaline.[17] The EHEC outbreaks have been reported from low-pH foods like bleached cake flour, with a pH of 4.4.[18] Bergholz and Whittam[19] compared the acid resistance of O26, O111 and O157 and stated that O157 has more ability to survive in acidic conditions, which simulate gastric acidity. Thus, the observations of the present study corroborate with earlier studies that these pathogens can survive and remain viable in food products that have been considered too acidic for the survival of foodborne pathogens. It is to be noted that the survival of EHEC in different pH conditions is affected by other factors, including the constituents of the testing medium, strain variability, age of the cells, and prevailing physico-chemical factors.

Elhadidy and Álvarez-Ordóñez[10] reported a higher reduction of *E. coli* O157 under lower and higher salt concentrations. However, Smith and Fratamico[21] found that when STEC cells were dried on a paper disc exposed to 5% salt, the survival rate reduced but reached undetectable levels at salt concentrations of 10% and 20%. Consequently, different biological interventions have been employed to decrease the transmission of STEC in foods. The acidity produced by fermented foods inactivates the pathogens as well as preserves them. Nevertheless, multiple studies claimed that the process of fermentation was not sufficient to inactivate *E. coli* O157.[22,23] Modifications to fermentation processes, such as the use of higher concentrations of salt and a reduction in water activity, can inactivate the pathogens. The present study also concluded that the cell inactivation rate will be faster at higher salt concentrations. In food, applications of such higher values of salt are impractical; however, tested values were valuable in validating the mathematical equation proposed here to extrapolate the data and predict suitable storage conditions to eliminate the risk of pathogens.

The D-value, or decimal reduction time, is a term used to describe the thermal resistance of bacteria. It represents the time required at a specific temperature to achieve a 90% reduction in the number of a given bacterial strain. In the study, extreme stress conditions resulted in a lower D-value. The D-value is influenced by various factors, including the food matrix, the bacterial strain, and the intrinsic properties of the food itself. Understanding D-values is essential for designing effective thermal processing methods, as multiple factors can impact the D-values of different organisms.[25]

The low infectious dose of approximately 100 cells of EHEC O157 strains[26] is a significant factor that points to the critical need for an in-depth understanding of their survival dynamics in different food storage conditions. This understanding is pivotal for formulating targeted control and prevention strategies against EHEC contamination in the food supply chain. In the realm of food safety, accurate knowledge of EHEC O157 survival kinetics is essential for developing evidence-based approaches to mitigate contamination risks. In food safety validation studies, the death rate prediction models are generally reliable in developing processing methodologies to control the pathogens in food environments.[27] The recovery of viable cells, even after three months of incubation at various food

storage/preservation environments, is a concern in food safety.

The limitation of the model is that survival was assessed in a sterile laboratory matrix, and factors such as interaction with other indigenous bacteria in food are not considered here. A thorough validation of this model considering all biological, physical and chemical parameters and its combinatorial effect is suggested as an extension of this research that would reflect the real-state survival kinetics of the pathogen. Integration of insights from survival pattern studies and predictive modelling enables the customization of control strategies to specific food matrices and storage conditions, thus contributing to ongoing efforts to fortify the resilience of the food preparation and supply chain and protect public health.

Conclusion

A comprehensive understanding of the survival dynamics of the bacterium *E. coli* O157:H7 under various physicochemical conditions related to food is essential for effectively controlling this pathogen in food. In this study, the survival rates of an EHEC strain were evaluated under a range of stress conditions, revealing their ability to persist in ground bovine meat, particularly within acidic environments. Such predictive models are valuable for microbiological risk assessment and control of pathogens in food. However, limitations, such as the utilization of a single strain as a representative of the pathogen and the consideration of a limited range of stress parameters, might restrict the generalizability of the findings. Despite these constraints, elucidating the factors governing *E. coli* O157:H7 persistence and resistance is important for evidence-based strategies to mitigate contamination risks within the food supply chain.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and, therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from other Sources

Not Applicable.

Author Contributions

- **Jomy Joseph:** Methodology, Experimentation and Data collection, Writing – Original Draft
- **Vinoj Muttuvancherry Narayanan:** Conceptualization, Mathematical modelling and Data analysis, and Editing
- **Sudha Kalyanikutty:** Conceptualization, Data Analysis, Writing and Editing

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