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Memory
With a view to obtaining a Master's degree
In Biological Sciences

Option: Fundamental and Applied Microbiology

THEME

How do the survival kinetics of *Escherichia coli* and Salmonella differ during poultry processing plants and thermal inactivation process?

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Before the jury composed of:

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الملخص

تعد السالمونيلا والإشريكية القولونية من أكثر مسببات الأمراض المنقولة بالغذاء شيوعًا والتي تسبب أمراضًا للإنسان في جميع أنحاء العالم، حيث تعتبر لحوم الدواجن المصدر الرئيسي للتلوث. وتؤثر كل مرحلة من مراحل سلسلة إنتاج الدواجن، من المزرعة إلى المائدة، على انتشار مسببات الأمراض هذه. على الرغم من استراتيجيات التحكم المختلفة لتحسين الجودة الميكروبية لمنتجات الدواجن، إلا أن القضاء التام على هذان النوعين من البكتيريا لا يزال بعيد المنال. كان الهدف من هذه الدراسة هو توليد رؤى جديدة حول انتشار السالمونيلا والإشريكية القولونية خلال المراحل المختلفة لإنتاج لحوم الدجاج. بالإضافة إلى ذلك، هدفت الدراسة إلى التنبؤ بمصير هذان النوعين من البكتيريا عبر سلسلة الإنتاج حتى النقطة التي تسبق الاستهلاك مباشرة من خلال فحص تأثيرات درجات الحرارة المختلفة ومستويات الأس الهيدروجيني باستخدام أدوات النمذجة التنبؤية. تم جمع 27 عينة من المسلخ في خمس خطوات شديدة الاهمية ومن محلين جزارة. تم أخذ أكثر من 16000 قراءة لدرجة الحرارة و92 قياسًا للأس الهيدروجيني أثناء أخذ العينات ومن معدات التخزين البارد المخصصة للحوم الدجاج لمراقبة تقلبات هذه المعايير. كان لحم الدجاج ملوثا بالسالمونيلا والإشريكية القولونية عند عدة ظروف متساوية الحرارة (5، 25،30، 37 درجة مئوية). ثم تم دمج النتائج في النماذج الأولية (Logistic, Gompertz and Baranyi). تم استخدام مقاييس جودة الملاءمة مثل (R^2, RMSE and AIc) للمقارنة بين هذه النماذج الأولية. وبناء على هذه المعايير، وصف نموذج Gompertz بيانات النمو على أنها الأفضل، يليه نموذج Baranyi. تمت بعد ذلك نمذجة معدلات النمو القصوى التي تم الحصول عليها من كل نموذج أساسي كدالة لـ Tºc باستخدام Ratkowskyالمعدل وتم استخدام مفهوم جاما للتنبؤ بتأثيرات T°C و pHعلى نمو الميكروبات. أشارت نتائج التحليل الديناميكي إلى أن معدلات النمو النوعي الأمثل (μ_{opt}) للسالمونيلا والإشريكية القولونية كانت 1.63±0.08 1/ساعة و1.05±0.06 1/ساعة، على التوالي. بالإضافة إلى ذلك، واستنادًا إلى درجات الحرارة المسجلة الفعلية وقيم الأس الهيدروجيني، وجدت هذه الدراسة أن أعداد السالمونيلا والإشريكية القولونية (N_{max}) يمكن أن تصل إلى 8.9 لوغاريتم CFU/g و8.5 log CFU/g ، على التوالي، بعد 41 ساعة، بافتراض وجود رقم أولي. عدد السكان (N_0) من 1 سجل .CFU/g أظهرت النتائج أن T°C كان لها تأثير معنوي على نمو البكتيريا، خاصة أثناء الذبح عندما كانت T°C قريبة من .($\gamma T = 0.96$) (T_{opt}) في المقابل، كان لدرجة الحموضة تأثير ضئيل على نمو البكتيريا. إن فهم هذه العوامل يمكن أن يساعد في اتخاذ التدابير المناسبة لمنع التلوث. تبريد الدجاج عند درجة حرارة 4 درجات مئوية أو أقل يؤدي إلى إبطاء نمو البكتيريا بشكل كبير. إن الطهي السليم يقتل السالمونيلا والإشريكية القولونية بشكل فعال، ويمكن لممارسات التنظيف المناسبة أن تمنع التلوث المتبادل. يمكن استخدام نتائج هذه الدراسة للتنبؤ بنمو وبقاء السالمونيلا والإشريكية القولونية وتقييم مخاطرها في الدجاج أو منتجات الدجاج الثانوية الأخرى المعرضة لنطاق درجة حرارة واسع نسبيًا أثناء التصنيع والتوزيع والتحضير.

الكلمات المفتاحية: الإشريكية القولونية، السالمونيلا، النمذجة الأولية، مفهوم غاما، درجة الحرارة، الرقم الهيدروجيني

Abstract

Salmonella and *E. coli* are among the most common foodborne pathogens causing human illnesses worldwide, with poultry meat being a primary source of contamination. Each stage of the poultry production chain, from farm to table, affects the prevalence of these pathogens. Despite various control strategies to improve the microbial quality of poultry products, complete eradication of these bacteria remains unattained. The objective of this study was to generate new insights into the distribution of Salmonella and *E. coli* throughout different stages of chicken meat production. Additionally, the study aimed to predict the fate of these bacteria across the production chain up to the point just before consumption by examining the effects of varying temperatures and pH levels using predictive modeling tools. A total of 27 samples were collected from the slaughterhouse at five critical steps, and from two butcher shops. More than 16,000 temperature readings and 92 pH measurements were taken during sampling and from cold storage equipment dedicated to chicken meat to monitor the fluctuations of these parameters. Chicken meat was contaminated with Salmonella and *E.coli* at several isothermal conditions (5, 25, 30 and 37°C). Results were then fitted into primary models (Logistic, Gompertz and Baranyi). Measures of goodness-of-fit such as R^2, RMSE and AIc, were used for comparison for these primary models. Based on these criteria, Gompertz model described growth data the best, followed by the Baranyi model. The maximum growth rates obtained from each primary model were then modeled as a function of TºC using the modified Ratkowsky and the gamma concept was used to predict the effects of the T°C and pH on microbial growth. The results of the dynamic analysis indicated that the optimal specific growth rates (μ_{opt})for Salmonella and *E. coli* were 1.63±0.08 1/h and 1.05±0.06 1/h, respectively. Additionally, based on the actual recorded temperatures and pH values, this study found that the populations of Salmonella and E. coli (N_{max}) could reach 8.9 log CFU/g and 8.5 log CFU/g, respectively, after 41 hours, assuming an initial population (N_0) of 1 log CFU/g. Results showed that T°C had a significant effect on bacterial growth, particularly during slaughter when the T°C was close to the (T_{opt}) ($\gamma T = 0.96$). In contrast, pH had a minimal effect on bacterial growth. Understanding these factors can help to act appropriately to prevent contamination. Refrigerating chicken at or below 4°C significantly slows down bacterial growth. Proper cooking effectively kills Salmonella and E. coli, and appropriate cleaning practices can prevent cross-contamination. The results of this study can be used to predict the growth and survival of Salmonella and *E. coli* and assess its risk in chicken or other chicken by-products exposed to a relatively wide temperature range during manufacturing, distribution and preparing.

Key words: E. coli, Salmonella, Primary modeling, Ratkowsky, gamma concept, Temperature, pH.

Résumé

Salmonella et *E. coli* sont parmi les agents pathogènes d'origine alimentaire les plus courants, responsables de maladies humaines à l'échelle mondiale. La viande de volaille constitue une source majeure de contamination. Chaque étape de la chaîne de production avicole, de la ferme à la table, influence la prévalence de ces pathogènes. Malgré diverses stratégies de contrôle visant à améliorer la qualité microbiologique des produits avicoles, l'éradication complète de ces bactéries reste inatteignable. L'objectif de cette étude était de générer de nouvelles connaissances sur la distribution de Salmonella et d'*E. coli* à différentes étapes de la production de viande de poulet. De plus, l'étude visait à prédire le sort de ces bactéries tout au long de la chaîne de production jusqu'au point précédant la consommation en examinant les effets des variations de température et de pH à l'aide d'outils de modélisation prédictive. Au total, 27 échantillons ont été collectés à l'abattoir à cinq étapes critiques ainsi que dans deux boucheries. Plus de 16 000 relevés de température et 92 mesures de pH ont été effectués lors des prélèvements et à partir des équipements de stockage frigorifique dédiés à la viande de poulet afin de suivre les fluctuations de ces paramètres. La viande de poulet a été contaminée par Salmonella et *E. coli* sous plusieurs conditions isothermes (5, 25, 30 et 37°C). Les résultats ont ensuite été intégrés dans des modèles primaires (Logistic, Gompertz et Baranyi). Des mesures d'adéquation telles que R^2, RMSE et AIC ont été utilisées pour comparer ces modèles primaires. Sur la base de ces critères, le modèle Gompertz a décrit le mieux les données de croissance, suivi du modèle Baranyi. Les taux de croissance maximaux obtenus à partir de chaque modèle primaire ont ensuite été modélisés en fonction de la température (Tºc) à l'aide du modèle de Ratkowsky modifié. Le concept gamma a été utilisé pour prédire les effets de la température et du pH sur la croissance microbienne. Les résultats de l'analyse dynamique ont indiqué que les taux de croissance spécifiques optimaux (μ_{opt}) pour Salmonella et *E. coli* étaient respectivement de 1,63 ± 0,08 1/h et 1,05 ± 0,06 1/h. De plus, sur la base des températures et des valeurs de pH réellement enregistrées, cette étude a révélé que les populations de Salmonella et d'*E. coli* (N_{max}) pourraient atteindre respectivement 8,9 log CFU/g et 8,5 log CFU/g après 41 heures, en supposant une population initiale (N_0) de 1 log CFU/g. Les résultats ont montré que la température avait un effet significatif sur la croissance bactérienne, en particulier lors de l'abattage où la température était proche de la température optimale de croissance (T_{opt}). En revanche, le pH avait un effet minime sur la croissance bactérienne. Comprendre ces facteurs peut aider à prendre des mesures appropriées pour prévenir la contamination. La réfrigération du poulet à une température inférieure ou égale à 4°C ralentit considérablement la croissance bactérienne. Une cuisson appropriée permet d'inactiver efficacement Salmonella et *E. coli*. Les résultats de cette étude peuvent être utilisés pour prédire la croissance et la survie de Salmonella et d'*E. coli*, et évaluer leur risque dans le poulet ou d'autres sous-produits de poulet exposés à une large plage de températures durant la fabrication, la distribution et la préparation.

Mots clés: *E. coli*, Salmonella, Modélisation primaire, Ratkowsky, concept gamma, Température, pH

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At the end of this memory,

First of all, we would like to thank Allah, the Almighty, the Merciful, for

having given us the strength, the courage and above all the patience to accomplish this work.

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Dedication

First, we thank Allah for everything for courage, success, and patience.

I dedicate this work:

To my mother, the most beautiful mother, who is the architect of my life. You showed me the right path to success. And to my father, thank you for your sacrifices, understanding, and advice.

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Zed El Kheir Imane

Dedication

I begin by giving thanks to Allah and his goodness, for the patience, skill and courage he has given me to get to this point.

With all my eternal love and with the intensity of my emotions. I dedicate this modest end-of-study work to my dear father, may Allah have mercy on him, who has always been available and ready to help me in my academic journey, who has ensured that I have arrived here. This work is the fruit of the sacrifices you made for my education and training. I affirm my deep affection and respect. You are a source of pride for me.

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Atlaoui Rim

Abreviations list

E.coli: *Escherichia coli.*

TSI: triple sugar iron fermentation.

SS: Salmonella-Shigella.

MM: Mannitol-Motility agar.

RSS: sum of squares.

MSE: mean square error.

RMSE: root mean square error.

AIC: Akaike's Information Criteria.

BIC: Bayesian information criteria.

EV: Evisceration.

CS: Carcasses after evisceration.

EC: After scalding.

AB: Slaughter.

O: scalding water.

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General Introduction

Poultry meat is the main type of meat consumed in many countries around the world, including Algeria. It has important role in human nutrition as it is desirable foodstuff. It is important source for protein, fat, essential amino acids, minerals, vitamins and other nutrients (Biesalski, 2005). On the other hand, they are considered an ideal culture medium for growth of many organisms because of their high moisture, high percentage of nitrogenous compounds of various degree of complexity, plentiful supply of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) of a favorable pH for most of enteric micro-organisms (Mohammed, 2011)

On a global scale, Salmonella, Campylobacter and *E.coli* has been estimated that the majority of epidemics of bacterial origin involve chicken as the means of transmission (Greig and Ravel 2009). This constitutes a major concern in the poultry industry (Corrégé, 2001) despite the importance of the poultry industry in Algeria, data on the prevalence of Salmonella and *E.coli* in broiler farms are very limited. Salmonella and *E. coli*, as members of Enterobacteriaceae, are considered the 2nd most common serious causes of foodborne illness worldwide (FSIS, 2008).

Escherichia coli is a bacterium that exists naturally as part of the normal gut flora of healthy humans and other mammals. The majority of strains are harmless and do not cause any food poisoning problems; however, there are a few strains of this organism which are pathogenic to humans and are associated with food related illness. *E.coli* O157 was first isolated from foods in Canada in 1974 and was recognized as a foodborne pathogen in 1982 (Riley et al. 1982). Salmonella serovars are capable of infecting a wide range of domestic animals which include sheep, cattle, poultry and pigs. Salmonella are the most common causes of foodborne illness worldwide, and the two foods that are most commonly associated it are eggs and poultry meat (Dar et al, 2017)

Those two pathogens can gain access to chicken meat through the whole cycle of production starting with scalding, defeathering and evisceration besides cross-contamination which may come from adjacent birds and contaminated equipment. Throughout chicken slaughtering and preparation steps, fecal matter may contaminate carcasses due to evisceration faults (Mbata, 2005).

Recent studies have shown relations between microbes and environmental factors, such as pH (Fierer and Jackson 2006; Lauber et al. 2009; Hartman et al. 2008) and temperature variation, they are ones of the most important stress factors for the existing microorganisms in the environment (Noor et al, 2012). Temperature is the major environmental factor that is likely to

change during processing and storage. Therefore, dynamic models have been developed to predict the growth of bacteria in foods under changing environmental factors. (V.K. Juneja et al. 2007)

Analyzing hazards and implementing preventive measures are necessary steps to control health safety. To do this, quantitative criteria must be sought in order to assist in the decision. Until now, the main obstacle has been the difficulty in quantifying microbial growth in foods. On the basis of predictive microbiology, criteria for calculating growth potential are presented. Associated with microbiological expertise they make it possible to predict this growth. Traditional microbial enumeration techniques are time consuming and therefore, mathematical models are being developed for predicting microbial growth. Predictive microbiology is a combination of mathematical modeling and bacterial growth/inactivation responses to several factors, such as temperature, pH, and water activity (Membre et al., 2005). It is a basic tool for estimating microbial behavior in foods during processing and storage (Bovil et al., 2001).

Therefore, the aims of the current study were to generate new data on the situation of Salmonella and *E. coli* at the steps of the poultry slaughter process. The primary objective of this study is to highlight the current occurrence of Salmonella spp. and E. coli in raw chicken meat throughout the production chain. The second part of this work is dedicated to modeling the growth and/or inactivation of these two pathogens in response to the variation of two environmental factors throughout the production chain: temperature and pH. This is done, on one hand, to assess the level of compliance with good hygiene practices and their impact on the hygienic quality of chicken meat, and on the other hand, to qualitatively estimate the risk these pathogens pose to the population.

This document is structured around the following axes: a bibliographic section that presents chicken meat, Salmonella, and *E. coli*, along with their various virulence factors; a section on the methodology adopted for this work; and subsequently, the results of this study will be discussed. The discussion will pave the way for a conclusion that summarizes the findings of this work, along with perspectives, recommendations, and suggestions for future studies.

Bibliographic synthesis

1. Definition of meat

Meat is the flesh of animals used for human food. According to the World Organization for Animal Health, meat is any edible part of an animal and considers the word "animal" to be any mammal or bird. This vocabulary includes the flesh of mammals (sheep, cattle, goats, camels, etc.), birds (chicken, turkey, guinea fowl, etc.) and fish. Meats are divided according to color into: red meats and white meats and according to fat content into: Lean meats and meats more or less rich in fat. (Benyoucef and Bouzegag 2006)

2. Definition of chicken meat

White meat, called “poultry meat, generally includes all products, beginning from carcasses to edible meats, including cutting and processing products currently marketed in various forms (Bourgeois et al., 1988), the Codex also defined white meat as the edible part of any domestic bird, including chickens, turkeys, ducks, geese, guinea fowl and pigeons, killed in slaughterhouses. (Codex alimentaires, 2015).

The chicken belongs to the species *Gallus Gallus,* order Galliformes. It has become a domestic fowl since the dawn of time, and has adapted well to the company of man. (Yves, 2009)

Poultry farming has grown significantly in many countries, becoming the main livestock production in terms of the volume of meat produced and the amount of compound feed used. At the same time, the consumption of poultry products has steadily increased for this type of production, partly explained by the fact that chicken meat from livestock farming has increased, without being limited by religious prohibitions or culinary traditions. In addition, chicken meat is less expensive than other meats. (Larbier and Leclercq, 1992)

3. Global poultry meat market

In 2019, poultry meat represented around 39% of global meat production. To meet growing demand, global poultry meat production increased from 9 to 132 million tons between 1961 and 2019. The FAO and OECD agricultural outlook expect an increase in poultry production by 2.3% per year from 2019 to 2023 (134.5 MT forecast in 2023), mainly to respond to changing dietary preferences. While the production of all meats combined would only increase by 1.6% per year. (OECD/FAO, 2014; Benamar, 2022)

Americans are the largest consumers of poultry meat with 49.8 kg per capita per year. Consumption in the European Union (EU) reached 23.4 kg per capita per year (Faostat, 2015). The

main global exporters of chicken meat are Brazil and the United States while the main importers are Hong Kong and China. (Faostat, 2015; Benamar, 2022)

4. Algerian poultry meat market

In Algeria, white meats and particularly those from broiler chickens contribute to the supply of proteins although their consumption is low 6.6 kg/inhabitant/year in 2014 and estimated to be 7.6 kg/inhabitant/year in 2023 (OECD/ FAO, 2014). Furthermore, according to Betraoui (2021), current consumption levels are estimated at 15 kg per inhabitant per year. Certainly, the current statistical data are much higher if several factors are taken into consideration, especially the increase in production on the one hand and even a small increase in the standard of living on the other. (Benamar, 2022) (**figure 1**)

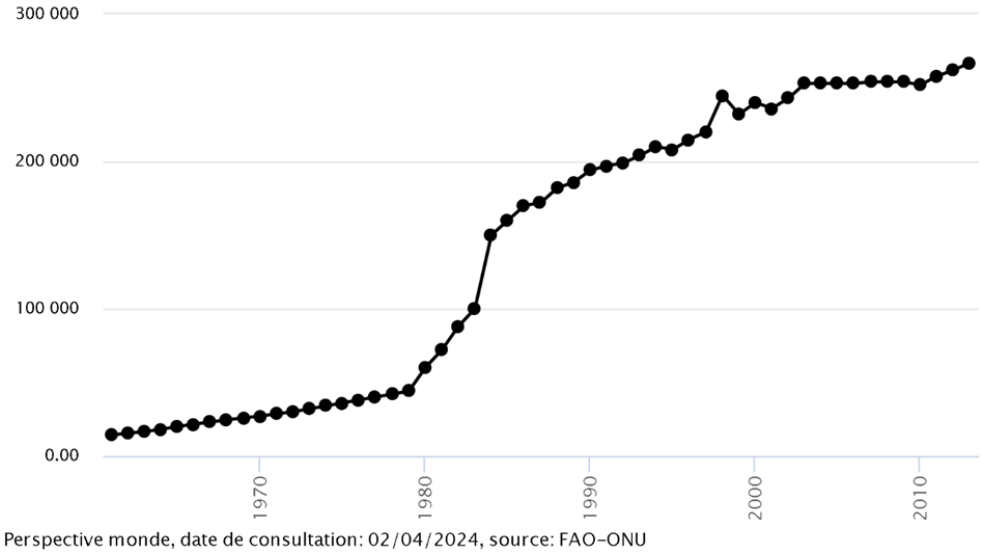


Figure 1: food production: meat (chicken) (tonnes), Algeria

5. The chemical composition of chicken meat

Meat occupies an essential place in the human diet. Chicken meat in particular, is an excellent source of protein and B vitamins, and is also rich in nutrients, trace elements and minerals (Brunel, 2019), It is essentially made up of: (**Table I**)

Table 1: chemical composition (g) and energy values (kcal) for 100g of edible fraction of chicken meat. (CIV ,2010)

Composite	Water (g)	Protides (g)	Lipides (g)	Glucides (g)	Energy (kcal)
Content	72.7	21	5.6	Traces	138

5.1. Water

According to Frenot and Vierling (2001), water is the most important component, both quantitatively and functionally. It represents 72% in muscle.

5.2. Proteins

Chicken is an excellent source of protein, 40% of amino acids are essential amino acids (Gandermer, 1992). According to the meat information center CIV (2010), the advantage of meat protein is of very good quality because they contain all the essential amino acids in balanced proportions and they are well assimilated by the body. (See **table II**)

Table 2: Amino acids content of chicken proteins. (Larbier et Leclercq, 1992)

Amino acids	Cysteine	Histidine	Arginine	Tyrosine
Content (mg/100)	1,3	3,5	6,8	4 ,3

5.3. Lipids

The comparison between poultry meat and different meats shows that poultry meat is less fatty than other meats (FISA, 2010). According to Larbier and Leclercq (1992), the body fat percentage in chicken is 17%. The percentage of fat in chicken varies depending on the anatomical origin of the piece and the degree of trimming.

5.4. Carbohydrates

Poultry muscles do not contain carbohydrates (Favier, et al., 1995). While CIV, (2010), reports the presence of trace carbohydrates in chicken meat.

5.5. Vitamins

Chicken meat is an important source of vitamins essential for the development of the living organism and according to Watier (1992), chicken meat is rich in group B vitamins (**Table 3**).

Table 3: The main vitamins in chicken meat.

Vitamins	Content
Ascorbic acid (c)	2,5 Mg
Thiamine (B1)	0,10 Mg
Riboflavine (B2)	0,20 Mg
Pyridoxine (B6)	0,5 Mg
Folic acid (B9)	9 Ug
Coblamine	0,5 Ug
Nicotinic amide (pp)	7 Mg

5.6. Minerals

Chicken meat is rich in minerals, it contains on average 1 to 2 mg of iron for 100g of edible part, poor in calcium, but rich in phosphorus and potassium. (Frenot and Vierling, 2001) (**table 4**)

Table 4: Mineral salt content of chicken meat, content per 100g of edible parts. (Virling, 2003)

Element	Sodium	Potassium	Phosphorus	Calcium	Magnesium	Iron	Zinc
Content(mg)	80	350	200	12	37	1.8	0.85

6. Main steps of the slaughter chain in poultry slaughterhouses

The slaughter itself brings together a series of operations, which are:

6.1. bleeding

Bleeding is carried out by sectioning the jugular and the carotid artery. It allows the death of the animal and emptying the muscles of part of the blood they contain. This operation constitutes an important factor in meat preservation. However, whatever the mode of bleeding; only 50% of blood is eliminated. (FRAYSSE, 1990) (**figure 2**)

Figure 2: Chicken slaughter in Laghouat slaughterhouse (personal photos)

6.2. Scalding

This operation consists of dipping the chicken in a tank of hot water with a temperature of 51°C to 52°C. (**figure 3**) The high temperature helps prepare the skin and feathers for subsequent plucking operations. It is recommended to avoid temperatures above 52°C because the skin walls can tear off. In fact, the carcasses may undergo unwanted surface cooking. The scalding process is the first process step to reduce the number of pathogenic bacteria on carcasses. (Russell, 2003) Therefore, the scalding process was suggested as a critical control point (CCP) in the Hazard Analysis and Critical Control Points (HACCP) plan for broiler slaughterhouses. Because of the

high rates of broilers entering into the scalder, which could be as high as 200/min, bacterial cross contamination from one carcass to others is possible (Osiriphun et al. 2012).

Figure 3: Chicken scald in Laghouat slaughterhouse. (Personal photos)

6.3. Plucking

It consists of removing feathers while preserving the integrity of the skin. It is carried out between rotating drums to which rubber fingers are attached which strike the feathers and detach them. (**figure 4**) In some cases, there remain pinfeathers (feathers that are difficult to extract) which require finishing by hand.

Figure 4: Chicken pluck in Laghouat slaughterhouse. (Personal photos)

6.4. Evisceration

Thinning or partial evisceration This is an extraction of the intestine through the cloacal orifice and emptying of the crop which remains in place along with the gizzard, heart, liver and lungs. The total evisceration consists of the total removal of the esophagus, the crop, the trachea, one of the thoracic (heart, lungs) and abdominal viscera (gizzard, liver, intestines) as well as the section of the neck and paws.

6.5. Internal and external washing

Washing is primarily used to remove visible dirt and bloodstains and improve the appearance of the carcasses. It is done by washing the carcasses with drinking water. This operation makes it possible to improve the final product presentation and reduce the level of contamination.

7. Microflora of chicken meat

Meat is a favorable substrate for microbial development. It is an excellent growth medium due to its high nutrient content for many microbial species. Among the pathogenic germs which contaminate meat and are responsible for food poisoning are in general, bacteria of the species *Salmonella spp*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*, *Yersinia, enterocolitica*, *Shigella* and recently *E. coli* entero Hemorrhagic. (Sabrina and Hasna 2022)

7.1. Salmonella

Salmonella is straight, non-spore forming rods, medium-sized, measuring about (0.7 - 1.5 × 2.0 - 5.0um), Gram-negative, non-capsulated, nonacid fast, but cells can be stained readily with common dyes, such as methylene blue or carbol-fuchsin. They are facultatively anaerobic, can grow well under both aerobic and anaerobic conditions. They are chemoorganophilic, having both a respiratory and fermentative type of metabolism. Salmonella bacteria are using peritrichous flagella for their movement (except *S. pullorum* and *S. gallinarum*). Some Salmonella possess fimbriae (Rahman et al. 2018). (**figure 5**)

The classification of Salmonella has been controversial for many years, now its taxonomic classification depends on the Kauffman White scheme (1934) in which typing is primarily performed using serological identification of somatic (O), flagella (H) and capsular (K) or (Vi) antigens. According to most recent nomenclature, the genus Salmonella consists of two major species. (Rahman et al. 2018). According to Bergey's manual (2001) the genus Salmonella has been classified into:

- Domain : Bacteria
- Phylum : Psroteobacteria
- Class : Gammaproteobacteria
- Order : Enterobacterales
- Family : Enterobacteriaceae
- Genus : Salmonella

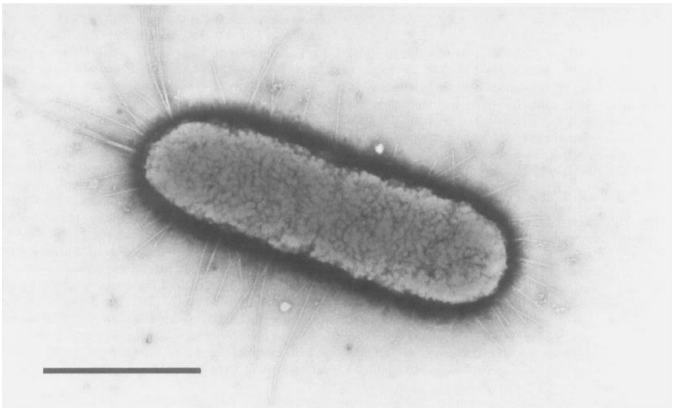


Figure 5: transmission electron micrograph of Salmonella. Bar 1 um.

7.1.1. Growth Requirements

Salmonella is facultative anaerobic and can grow well under both aerobic and anaerobic conditions. Temperature and pH are two factors commonly employed by food processors to control or inactivate pathogens and undesirable microorganisms in foods. (Doyle and Roman 1981) The optimum temperature to support Salmonella multiplication is 37 °C, but some growth is observed over a range from about 5 °C to 45 °C. Salmonella can grow within a pH range of approximately 4.0–9.0, with an optimum around 7.0, although cellular components such as flagella and fimbria may not be expressed under extreme pH conditions. The nutritional requirements of salmonella are relatively simple, and most culture media that supply sources of carbon and nitrogen can support their growth. The viability of Salmonella cultures can be maintained for many years in simple media, such as peptone agar or nutrient agar, which have been stab inoculated, sealed, and held at room temperature. (Gast and Porter 2020). Typical Salmonella colonies on agar media are about 2–4 mm in diameter, round with smooth edges, slightly raised, and glistening. (Gast and Porter 2020)

7.1.2. The virulence factors of Salmonella

Salmonella is one of the most frequently isolated food-borne micro-organisms. It is a major worldwide public health involvement, accounting for 93.8 million foodborne illnesses and 155,000 deaths per year. The genus consists of more than 2500 serological distinguishable variants in which more than half of them belong to *Salmonella enterica* subsp. *enterica*, which accounts for the majority of Salmonella infections in humans. Most of Salmonella serotypes are potentially pathogenic, causing sporadic infections, as well as outbreaks of fatalities, most of the *Salmonella* spp. associated to humans and other mammals' diseases are *from S. enterica subsp. enterica* with *S. Enteritidis and S. Typhimurium* were responsible for most of the infections (Dunkley et al.,

2009). Other Salmonella serovars are zoonotic or potentially zoonotic, while some usually found in the environment such as *Salmonella bongori, Salmonella enterica* subsp. *salamae, Salmonella enterica* subsp. *arizonae, Salmonella enterica* subsp. *diarizonae, Salmonella enterica* subsp. *houtenae* and *Salmonella enterica* subsp. *indica* are occasionally associated with human diseases (Shafini et al. 2017). While some are less pathogenic and causing minor infections in both human and most animal species (Rahman et al. 2018). Salmonella contamination in beef and chicken can occur at several stages along food supply chain includes productions, processing, distribution, retailing and also preparing and handling by consumers. The persistence of Salmonella in premises may include resistance of the specific strains to the disinfection, desiccation and biofilm production (Shafini et al. 2017).

The virulence factors involved in Salmonella infections are numerous and complex, which include:

- The ability to invade cells (contributing agents like surface polysaccharide O antigen, flagellar H antigen, and fimbriae).
- A complete lipopolysaccharide coat.
- The ability to replicate intracellularly.
- Elaboration of at least three toxins:

Endotoxin: It is a lipopolysaccharide of the wall of the bacteria, which can produce fever when released into the bloodstream of the infected individual.

Enterotoxin: It reduces a secretary response by epithelial cells that result in fluid accumulation in the intestinal lumen while it is thought that this toxin may not play a major role in diarrhea seen in Salmonella food poisoning.

Cytotoxin: Cause iron chelating structural damage to intestinal epithelial cells by inhibiting protein synthesis.

- Iron chelating proteins (siderophores and enterobactin of the bacteria) provide iron from the iron-binding proteins of the host necessary for bacterial growth.
- Several other virulence factors are known to contribute to the establishment of disease like adhesion pilli, production of colicin and porins, ability to resist the lethal effects of serum complement and large plasmids (30- 60 mega daltons). (Rahman et al. 2018)

7.1.2.1. The effect of salmonella on health

Human infection is usually attributed to cross-contamination in the kitchen, inadequate cooking, and improper storage temperatures (Silliker, 1980). Salmonellosis is caused by non-typhoidal

Salmonella enterica serotypes (serotypes other than *S. Typhi* and *S. Para- typhi*) and is typically characterized by a self-limiting gastro- enteritis syndrome (manifested as diarrhea, fever and abdominal pain), with an incubation period between 4 and 72 h and mortality being rare. In healthy humans, the infectious dose is generally 10^6 to 10^8, but lower bacterial counts can cause disease in certain conditions, as well as in infants and the elderly. Although uncommon, life-threatening invasive infections with bacteremia (5%–10% of infected persons) and/or other extra-intestinal infections may occur, affecting especially the risk groups (infants, young children, older people and immunocompromised patients). In severe cases, effective antimicrobial agents are essential, so the emergence of Salmonella that are resistant to critical antibiotics is of concern. In industrialized countries, the main reservoir of non-typhoidal Salmonella is the intestinal tract of food-producing animals, which readily leads to contamination of diverse foodstuffs. Therefore, despite other sources (e.g. contact with animals/reptiles, environment or person to person), foodborne salmonellosis is the most relevant source with a high global impact in human health. It was estimated that non- typhoidal Salmonella causes around 93.8 million illnesses and 155 000 deaths each year worldwide. In the USA, the CDC estimated more than 1 million annual cases of foodborne salmonellosis; they were associated with the largest number of hospitalizations and deaths compared with other foodborne microbial agents. In Europe, salmonellosis has been the second most common zoonosis (82 694 confirmed cases and 20.4 cases per 100 000 population in 2013).

7.1.2.2. Typhoid

Human typhoid occurs following the ingestion of *S. enterica* serovar Typhi bacteria, usually from contaminated water or animal products or close contact with an infected individual or carrier. Salmonella is capable of infecting a wide variety of cells including DCs, macrophages, hepatocytes, neutrophils, colonocytes and other epithelial cells. In vitro, within minutes of contact with cells. In humans, typhoid disease manifests one to 2 weeks following bacterial inoculation with generalized fever and malaise, abdominal pain with or without other symptoms including headache, myalgias, nausea, anorexia and constipation. Diarrhea occurs occasionally but is typical only of infection in the immunocompromised. Hepatosplenomegaly is common but not present in all cases and diffuse abdominal tenderness is usual. Fever is typically mild at first and worsening as disease progresses (Coburn, Grassl, and Finlay 2007).

7.1.2.3. Antibiotics resistance

The emergence of Salmonella with antimicrobial resistance is mainly promoted by the use of antibiotics in animal feed to promote the growth of animals, and in veterinary medicine to treat bacterial infections in those animals. (Hyeon and al. 2011) this poses a high risk of zoonotic disease with the transmission of Salmonella stains from animals to humans via the ingestion of food or water contaminated with the animals' faeces, direct contact or the consumption of infected food animals (Holmberg and al, 1984).

7.2. Escherichia coli

E. coli is the type species of the genus Escherichia, bacillus with rounded end, Gram-, measures approximately 2 to 4µm in length by 0.6µm in width, possessing neither capsule nor spores, it appears isolated or in short chains, and in some cases, in the form of very long filaments. Provided with cilia, it is generally mobile thanks to peritrichous ciliature but this mobility is very variable depending on the environment where the strain was sown. (Bey F. 2009) (**figure 6**)

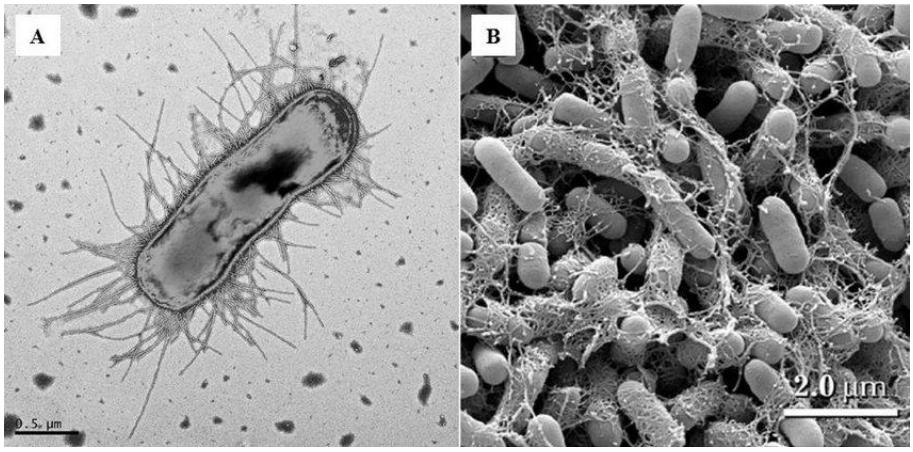


Figure 6: micrographs of *E.coli* by electron microscopy (A) one cell of *E.coli* and (B) of *E.coli* biofilm grown on glass coupons. (Bey F. 2009)

According to Bergey's manual (2012) the species *E.coli* has been classified into:

- Domain : Bacteria
- Phylum : Proteobacteria
- Class : Gammaproteobacteria
- Order : Enterobacterales
- Family : Enterobacteriaceae
- Genus : Escherichia
- Species: *Escherichia coli*

7.2.1. Growth Requirements

E. coli are straight rods, aerobes and facultative anaerobes; ferment most sugars producing gas but do not produce H2S on TSI agar slants (A/A with gas). They are indole positive, methyl red positive, Voges Proskaur negative, simmon's citrate negative, catalase positive and urease negative (Soomro et al., 2002). According to the modified Kauffman scheme, *E. coli* serotaxonomy is based on their antigenicity O (somatic), H (flagellar), and K (capsular) surface antigen profiles. (Nataro and Kaper, 1998). *E. coli* strains can survive and multiply when stored between 0 ºC, 6 ºC and 12 ºC; and in dry foods with a wide range of water activity and pH values. (Samedpour and Liston, 1994). It develops in 24 hours at 37°C on agar media, giving round, smooth colonies with regular edges, 2 to 3 mm in diameter, non-pigmented on blood agar. *E. coli* colonies are smooth, dull gray, and 2 to 3 mm in diameter. On Mac Conkey medium, Lactose-positive *E. coli* colonies are pink to red in color, flat, dry and 2 to 3 mm in diameter, they are generally surrounded by a darker pink zone of precipitated bile salts. Lactose-negative *E. coli* colonies produce colorless colonies on Mac that are 2 to 3 mm in diameter. (Haouzi R. 2013)

7.2.2. The virulence factors of E. coli

Many different types of foods are sources of the bacterium and have been identified as a potential source of Shiga Toxin-producing *Escherichia coli* (STEC) for which such raw or undercooked foodstuffs get contaminated either during primary production (e.g. slaughtering) or further processing and handling (e.g. cross contamination during processing, human-to-food contamination via food handlers). *E. coli* has been isolated worldwide from poultry meat (Canton et al. 2008; Adesiji et al. 2011). During the slaughter of poultry birds, there can be fecal contamination of the carcasses from the gut of these birds which means bacteria present in the spilled gut content is passed on as contaminants. Of importance is the coliforms especially Escherichia coli (Panisello et al. 2000). *Escherichia coli* strains are one of the normal bacterial floras of the gastrointestinal tract of poultry and humans. Ten to fifteen percent of the intestinal coliforms in chicken are of pathogenic serotypes (Barnes et al., 1997). Most *E. coli* are normal components of the intestinal flora, but certain pathogenic strains are an important cause of disease. Pathogenic strains differ from commensals in that they express virulence factors molecules directly involved in pathogenesis but ancillary to normal metabolic functions which disrupt normal host physiology and thereby cause disease (Donnenberg and Whittam, 2001).

Virulence factors of *Escherichia coli* are of two main types; those produced on the surface of the cell and those produced within the cell and then exported to the site of action. Those on the surface include different sorts of fimbriae that have a role in adhesion to the surface of host cells

but may also have additional roles such as tissue invasion, biofilm formation or cytokine induction. The activities of cell wall components are discussed and several exported virulence factors are described that have anti host cell activities. Others virulence factors enable the bacteria to grow in an environment of iron restriction. (L. Emo″dy and al. 2003)

7.2.2.1. The effect of *E.coli* on health

During the past two decades, severe outbreaks of gastrointestinal illness have occurred by food borne pathogenic *E. coli*, especially 0157:H7 (Armstrong et al. 1996). Among the diseases some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children (Diani et al. 2005) and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coli granuloma, and colibacillosis (Gross 1994).

7.2.2.2. Antibiotics resistance

Antibiotics have been successfully used in poultry for different purposes such as growth promotion, prophylaxis, or therapeutics. However, their indiscriminate use caused increased bacterial resistance, mainly in Escherichia strains (Hamilton L.S and al, 2006). The majority of *E. coli* recovered from retail meat and poultry have been found resistant to at least one antimicrobial, and an increasing proportion have been found resistant to clinically important, frontline antimicrobials, including trimethoprim-sulphamethoxazole, fluoroquinolones, and thirdgeneration cephalosporins (Carl M and al, 2004).

8. The effect of Temperature and pH on microbial growth

In recent years, interest in developing mathematical models to describe the growth of microorganisms has increased, especially in the fields of medicine and food science. The advantage of such models is that they can be used to simulate the effects of different environmental conditions on growth kinetics. Temperature and pH are the major environmental factors that affect growth which are studied most because of their importance in fundamental research (taxonomy, microbial metabolism) and their practical importance (control of bioprocesses in biotechnology and safe handling of goods, especially in the agriculture and food industries) (ROSSO, et al,1994).

9. Modeling in microbiology

Predictive microbiology makes it possible to better understand and control contamination levels in at-risk foods (McMeekin, 2002). Primary, secondary and tertiary models are used to model the behavior of microorganisms. The primary models consist of monitoring the evolution of the

concentration of microorganisms over time in a given environment. Secondary models model the behavior of microorganisms. The primary models consist of monitoring the evolution of the concentration of microorganisms over time in a given environment. Secondary models make it possible to describe the influence of environmental factors on the primary parameters. Models' concentration of microorganisms over time in a given environment. Secondary models make it possible to describe the influence of environmental factors on the primary parameters. Tertiary models use expert systems and databases to link primary models and describe the influence of environmental factors on primary parameters. Tertiary models use expert systems and databases to link primary and secondary models. Many growth kinetic models have been described in the literature, such as the Gompertz, log-linear, vitalistic, and Baranyi models. These models may be fit to observed data and used for statistical inference about growth and death rates in microbial populations of interest. (Payne et al. 2007)

9.1 Primary models

Models describing the development of numbers of organisms as a function of time (both for growth or inactivation) are called primary models. The microbial growth curve (as log numbers) typically has a characteristic sigmoid nature, with an apparent slow phase followed by a more rapid increase followed tools for microbiological risk assessment by a slowing down, finally reaching a maximum population level. A variety of models can be used to
fit the curve, such as the logistic, Gompertz, Richards, Schnute and Stannard model (Zwietering *et al.*,1990); the Baranyi model (Baranyi *et al.*, 1993; Baranyi and Roberts, 1994); and the three-phase linear (Buchanan *et al.*, 1997) models. The effectiveness of the chosen model is dependent not only on the ability of the model to reflect the observations but also on the purpose of modelling and the quality and type of data provided (Bassett et al,2012).

9.2 Secondary models

Primary models provide data on lag time, maximum growth rate and the maximum population reached from a given initial inoculum. As conditions change, these values change and secondary models attempt to produce mathematical formulae describing these changes, e.g. the effect of temperature on the lag time or the effect of pH on the specific growth rate. The aim of such modelling is to produce a model with the fewest parameters (parsimony), which describes the observed growth parameters so that predictions can be made (Ratkowsky, 1990). The main factors studied for their effects on growth in the primary models available are: Temperature, Acidity, Water activity… (Bassett et al,2012).

9.2.1 Temperature

Of all the hurdles to microbial growth in a foodstuff, temperature is one of the most critical. Ratkowsky *et al.* (1982) found that a square root expression fitted the observations of growth rate at temperatures below the maximum growth temperature. A distinct set of temperature models have been developed that are empirical in nature but that use the cardinal temperature values to provide a good fit to observed data having interpretable parameters (Rosso *et al.,* 1993), with cardinal parameters the minimum, maximum and optimum values for growth (Bassett et al,2012).

9.2.2 Acidity

For the effect of pH, exponential or square root models were initially developed, followed by Cardinal models (Rosso *et al.*, 1995).

9.2.3 Water activity

McMeekin et al. (1987) showed that there was a linear relationship between water activity (aw) and the maximum specific growth rate. Rosso and Robinson (2001) built on the success of the cardinal parameter models described previously by constructing a general model for the effect of water activity.

9.3 The Gamma hypothesis

The Gamma hypothesis (Zwietering et al., 1996) states that inhibitory processes affect microbial growth independently and that they combine together in a multiplicative manner, except where synergy or antagonism between factors occurs. Furthermore, the Gamma concept suggests that by understanding the effect of individual factors, each for example described by a Cardinal model, they can be combined in the form of "Gamma factors" or ratios of the observed growth to the uninhibited growth.

9.4 Predictive modelling tools

10.2.1. Pathogen Modeling program

http://pmp.arserrc.gov/PMPOnline.aspx .The Pathogen Modelling Program (PMP) is a package of models that can be used to predict the growth and inactivation of some foodborne pathogens under various environmental conditions. Predictions are based on specific papers, predominantly from US authors.

9.4.1 ComBase

www.combase.cc . ComBase is a database that comprises thousands of microbial growth and survival curves that have been collated in research establishments and from publications. They form the basis of microbial models presented in ComBase Predictor (a set of 23 growth models and six thermal death models for predicting the response of many

important food pathogenic and spoilage microorganisms to key environmental factors). ComBase also contains the Perfringens predictor (an application for predicting the growth of Clostridium perfringens during the cooling of meats), and the ability to fit predictive models to data defined by the user.

9.4.2. DMFit

www.ifr.ac.uk/safety/DMFit . DMFit is a software package (an Excel add-in) that can be used to estimate the specific growth rates from experimental data with the Baranyi model (Baranyi and Roberts, 1994). DMFit is part of the system used in-house at the Institute of Food Research to model the time variation of the logarithm of cell concentrations of bacterial batch cultures. It has been the main tool in the development of the models behind ComBase Predictor (see above).

9.4.3 Sym'previus

http://www.symprevius.net . Sym'Previus is a collection of tools for food safety inspections designed for food sector businesses to help strengthen HACCP plans, develop new products, better understand and quantify microbial behaviour, determine shelf lives and improve food safety.

9.4.4 Seafood Spoilage and Safety predictor

http://sssp.dtuaqua.dk . The Seafood Spoilage and Safety Predictor (SSSP) software has been developed to facilitate the practical use of mathematical models to predict shelf-life as well as growth of spoilage and pathogenic bacteria in seafood, and to evaluate the effect of constant or fluctuating temperature storage conditions.

9.4.5 Forecast

http://www.campden.co.uk/services/predictive-microbiological-models.pdf . Forecast is a predictive modelling tool developed by Campden BRI for growth of spoilage organisms in foods. It includes models for fish, meat, fresh produce and yeasts in fruits and drinks, plus a range of models relevant to acidified foods.

9.4.6 MLA refrigerator index calculator

http://www.foodsafetycentre.com.au/refrigerationindex.php . Meat and Livestock Australia (MLA) The refrigeration index (RI) is an index for the log growth of E. coli. It predicts the expected growth of E. coli on meat from temperature and other data. The central idea of the RI is to measure the performance of the chilling process until all the sites of microbiological interest are at or below 7ºC. The RI, as an indication of the effectiveness of refrigeration, is NOT a prediction of the number of E. coli in the product.

Methods and materials

1. Samples collection

In this study, 27 chicken samples were carried out as a total. Six samples of chicken meat were taken on February 19, 2024 from two different butcher's shop in the town of Laghouat and 21 samples from the slaughterhouse taken on February 26, 2024 from different stages of slaughter (5 after slaughter, 5 after scalding, 5 at the time of evisceration, 5 from carcasses after evisceration and one sample of the scalding water taken). At the same time of sampling, we measured the temperature and pH of these samples. Transportation of samples to the laboratory of Amar Thelidji University, Laghouat, was rapid and temperatures were between 10ºc and 20ºc and under sterile conditions. The following flowchart (**figure 8**) summarizes the plan for collecting these samples.

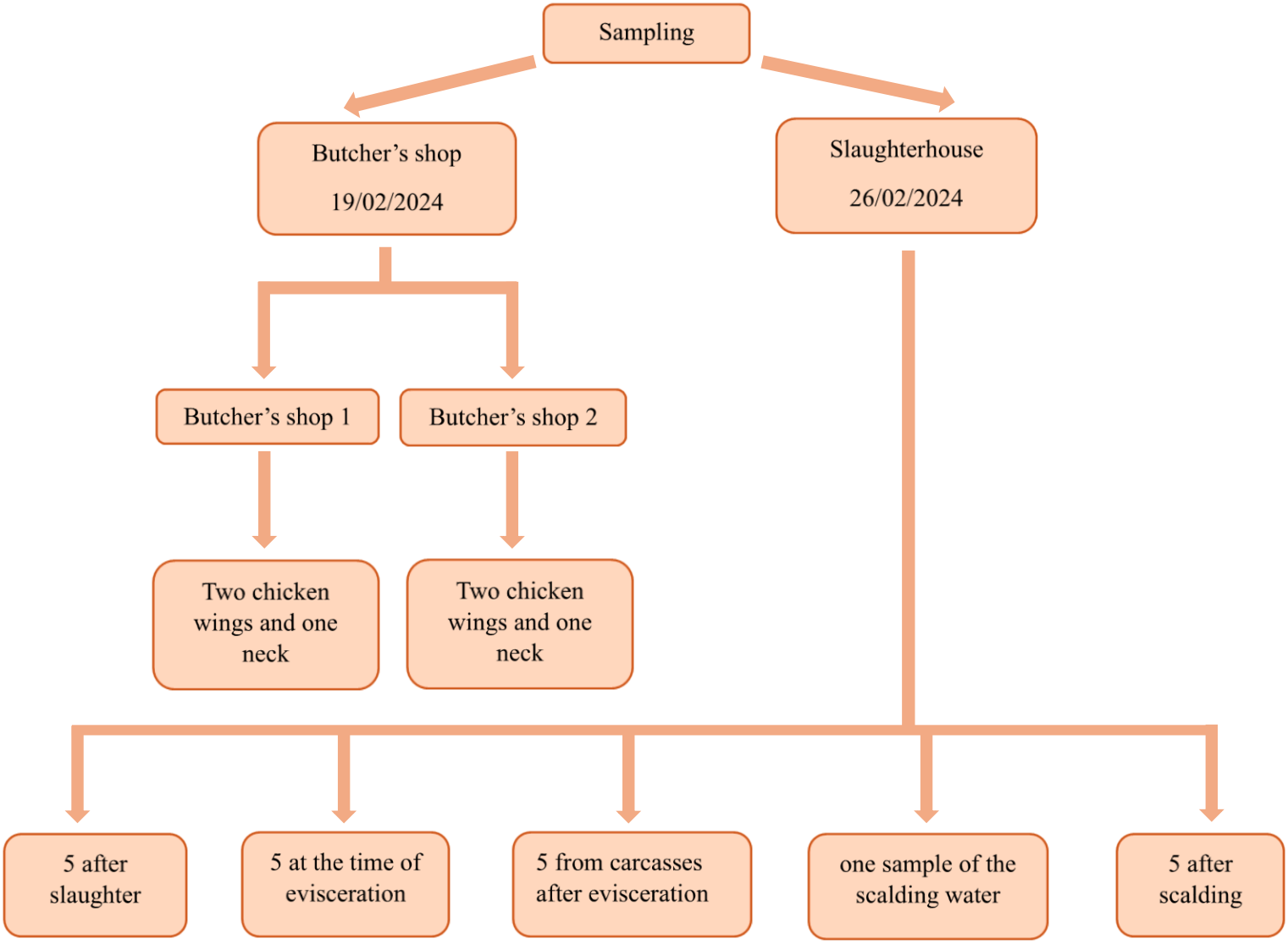


Figure 7: flow chart representing the samples collection.

Figure 8: collection of samples from Laghouat slaughterhouse (evisceration step).

2. Detection of Salmonella

The microbiological analysis for the search for Salmonella was carried out according to the standard (ISO 6579): 2002, in 04 steps: pre-enrichment to recover bacteria that have undergone stress; then a selective enrichment, favoring the multiplication of salmonella compared to flora competitor; followed by isolation on two specific selective media (SS, Hektoen), and two microscopic examinations by Gram coloration and methylene blue staining, and a biochemical identification.

2.1 Pre-enrichment

done in buffered peptone water (1 g of the sample in 9 ml of BPW), then incubated at 37°C for 24 hours. **(figure 7)**

Figure 7: Weighing samples to prepare them for the pre-enrichment step (photo taken in the laboratory).

2.2 Enrichment

For this, selenite – cystine broth is used. Using a sterile pipette, 1 ml of the pre-enrichment is taken, which is introduced into a test tube containing 9 ml of the selenite – cystine broth. We homogenized then incubated at 37°C for 24 hours. **(Figure 9)**

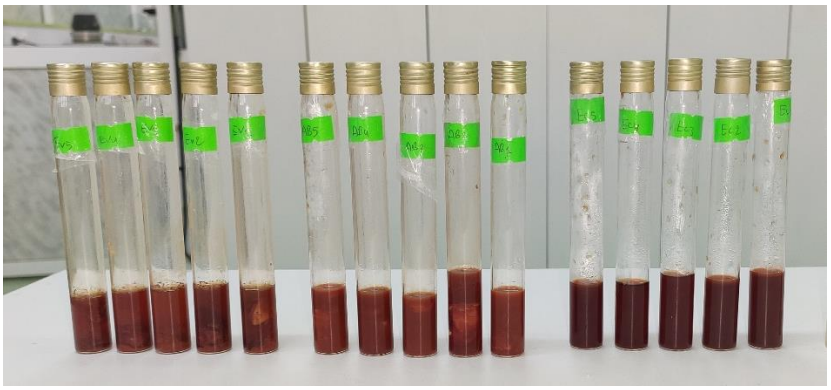


Figure 8: enrichment in selenite – cystine broth (photo taken in the laboratory*)*.

2.3 Isolation

Carried out on two Hektoen and Salmonella – Shigella SS agar media. 10µL is taken from the 10^{-3} decimal dilution selenite tubes seeded on the surface of SS agar **(figure 10)** and Hektoen **(figure 11)** agar in tight, spaced streaks. The petri dishes thus isolated are incubated at 37°C for 24 hours. Petri dishes considered positive are those presenting colonies with characteristics of Salmonella. On Hektoen medium, colonies rapidly ferment one of the 3 sugars (change from blue to salmon red) and/or produce H_2S (black center). On SS medium, characteristic Salmonella colonies (lactose negative) are opaque, translucent or transparent and usually with a black center (H2S positive). They are differentiated from Shigella colonies (lactose negative) which are colorless and coliforms which are pink-red (lactose positive). (**figure 11**)

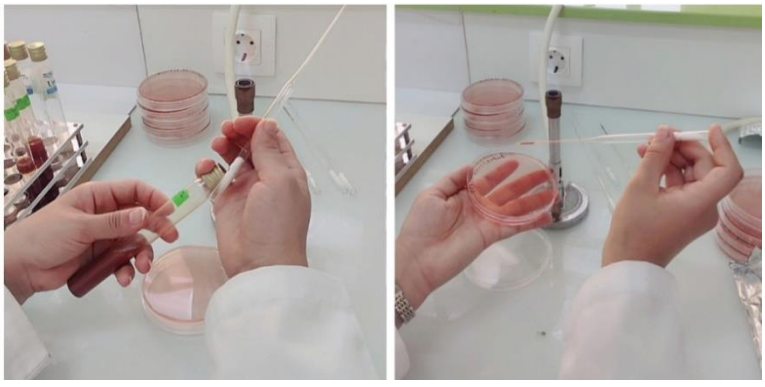


Figure 9: The isolation of salmonella strains on SS media (photos taken in the laboratory).

Figure 10: The isolation of salmonella strains on Hektoen medium

3. Identification of Salmonella

To identify micro-organisms down to the genus level, a series of tests are necessary. (Ludmila. B et al, 2023) Suspected colonies of Salmonella are purified on Hektoen agar and SS agar, the best stains were selected. These selected stains were later identified using macroscopic examination then microscopic examination (Gram staining and methylene blue) and biochemical tests which are: sugar fermentation using TSI, Mannitol motility test and the API ID 32E gallery.

3.1. Macroscopic examination

In nature bacteria often grow as colonies on surfaces. Observation of colony development and morphology has for many years been an important tool for identification and characterization of bacteria because individual species often form colonies of characteristic size and appearance. (HENRIK H and al, 2000) the colonies were observed with the naked eye. (Singleton, 1999)

3.2. Microscopic examination

Microscopic characteristics (size, shape, arrangement, color, gram reaction …) were observed according to the methods described in Manual of Methods for General bacteriology by American Society of Microbiology (ASM, 1981).

3.2.1. Gram staining

Gram staining was performed to observe size, shape, color, arrangement, gram reaction. In this method of staining a bacterial film is covered with a solution of one of the methyl violet dyes which is allowed to act for a definite period of time. The stain is then washed off and a dilute solution of iodine added. The iodine is allowed to react for a given period of time and then the film is treated with alcohol or acetone until almost all the dye is removed. Finally, a counterstain such

as safranin is added. Organisms that retain the methyl violet dye are called gram-positive organisms; those that fail to retain the primary stain but take the counterstain are called gram-negative. (B. B. BISWAS and al, 1970)

3.2.2. Methylene blue staining

This is a simple stain which is mainly used to demonstrate the presence of bacteria and to determine their gross morphology. For this reason, Loeffler's methylene blue is sometimes used as a counterstain to differentiate micro-organisms from background material. (J. R. Norris and D. W. Ribbons)

3.3. Biochemical identification

3.3.1. Mannitol motility test

Mannitol motility medium is bacterial growth medium used to detect the ability of bacteria to ferment mannite and produce nitrogen gas, and to indicate the motility of the organism. When inoculated with a sample organism and allowed to incubate, the medium will change color from red to yellow to indicate that the mannite has been fermented. The presence of bubbles in the medium indicates the presence of gaseous nitrogen, produced by the organism. The pattern of color change (localized along the inoculation site / dispersed throughout the medium) indicate the motility of the organism. (Myer and Koshi, 2001) **(figure 12).**

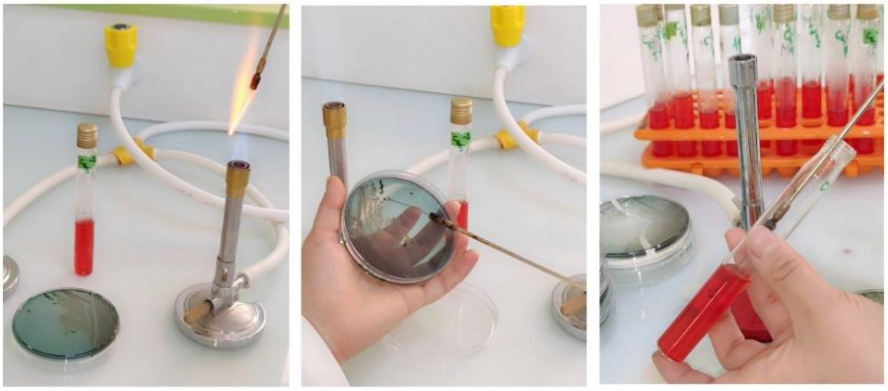


Figure 11: Mannitol motility test steps (photos taken in the laboratory).

3.3.2. Triple sugar Iron fermentation test

The Triple sugar Iron (TSI) test is a microbiological test roughly named for its ability to test micro-organism's ability to ferment sugars glucose, lactose and sucrose and produce hydrogen sulfide. It is often used to differentiate Enteric bacteria including Salmonella (D.M Rollins and S.W. Joseph, 2000).

TSI slopes with a butt of about 1 inch (3.5cm and 2.5cm) were inoculated by stabbing the butt and carefully streaking of slant using a sterile inoculating needle after slightly touching the center of a discrete colony on selective media. The tubes were incubated overnight at 35°C (**figure 13**), this test was done according to Barrow and Feltham (2003).

Figure 12: TSI test steps (photos taken in the laboratory).

3.3.3 Api ID 32 E plates

For the identification of salmonella genus, we used the Api ID 32E gallery, which is a standardized system for the identification of Enterobacteriaceae and other non-fastidious gram-negative bacilli, using 32 miniaturized biochemical tests, as well as a specific database. The reactions produced during the incubation period result in spontaneous color shifts or revealed by the addition of reagents. (**figure 14**) These reactions are read using the reading table, or identification software (Biomérieux, 2009), allowing identification of the species bacterial and biotype determination.

Figure 13: Api ID 32 E plates test (photos taken in the laboratory).

4. Conservation of Salmonella stains

After identifying the stains of Salmonella, their conservation is of paramount importance due to the long-term preservation of their characteristic morpho-cultural and biochemical properties. (Ludmila. B et al, 2023) (**figure 15**)

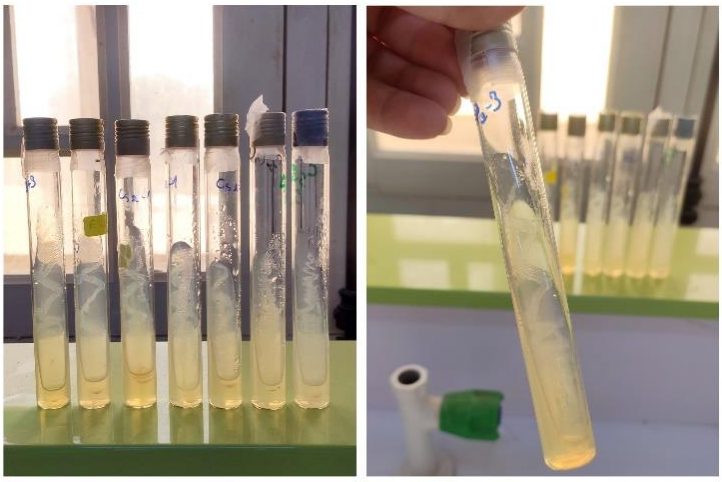


Figure 14: conservation of Salmonella stains in nutrient agar medium.

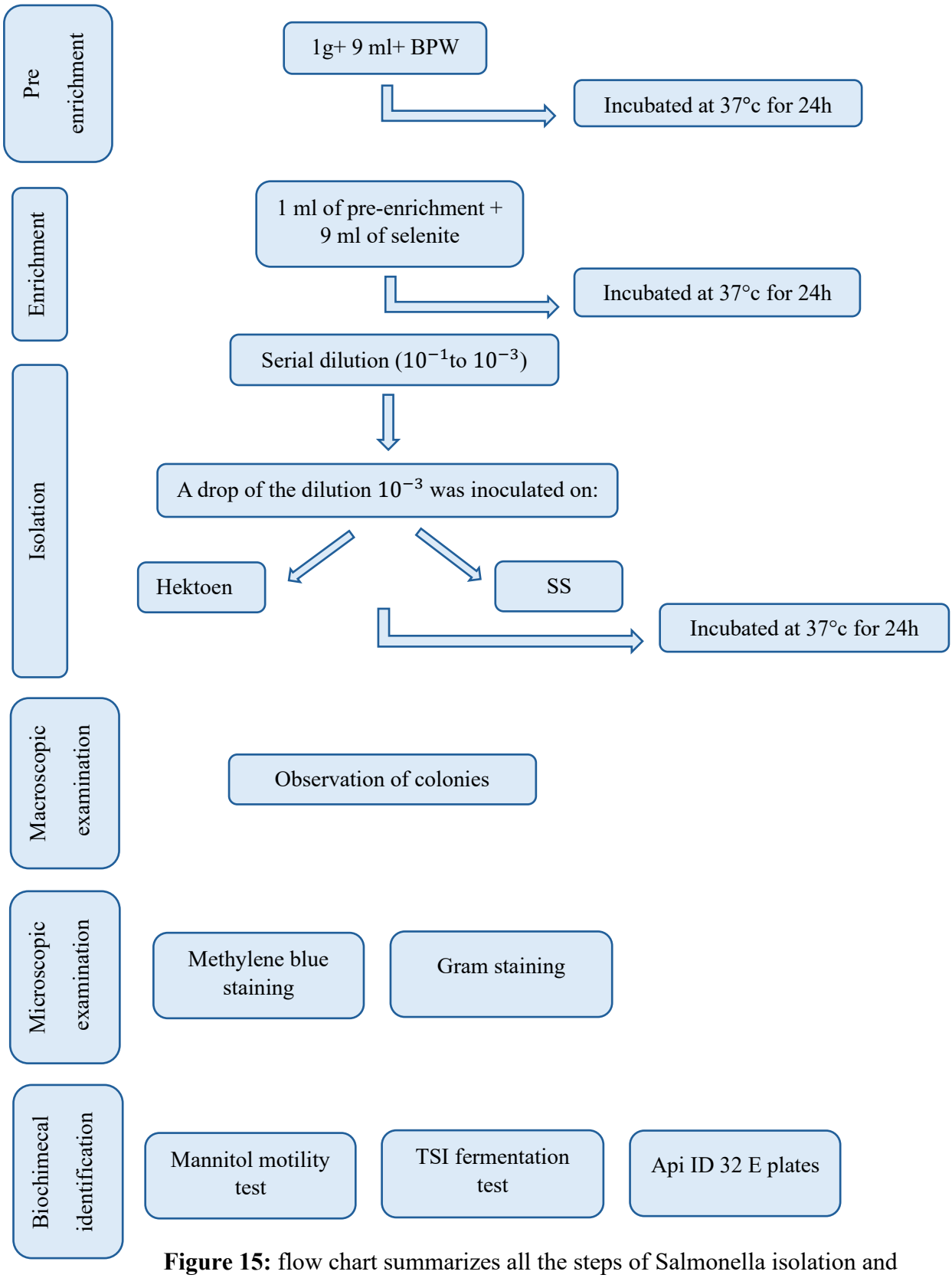


Figure 15: flow chart summarizes all the steps of Salmonella isolation and identification.

5. Growth validation kinetics of *E. coli* and Salmonella in chicken meat

In this study, we collected the temperatures measured at the slaughterhouse and various butcher shops, as well as the temperatures measured when storing chicken meat at home. This allowed us to compile a range of temperatures that chicken meat can encounter in the city of Laghouat, from slaughter to consumption. Four of these temperatures were selected: 5°C, 25°C, 30°C, and 37°C. These temperatures are the most critical and closest to the cardinal temperatures. These are the temperatures that were used in the contamination step, aimed at determining their effect on the growth of *E. coli* ATCC 25922 and two isolated strains of Salmonella (selected from the strains stored in the previous step) in chicken meat over different time periods. These times were carefully chosen to represent the average duration between slaughter and consumption of chicken meat in Laghouat (6h, 22h, 30h, 40h). They are also the most convenient for laboratory work and necessary for tracing the kinetics of bacterial growth.

5.1. Chicken fillet samples preparation

In this step we use the protocol of Ahmadi et al (2020) slightly modified. Fresh chicken fillet samples were locally purchased and promptly transported to the laboratory. Additionally, samples were obtained directly from the slaughterhouse. These samples were washed and aseptically cut into squared pieces weighing 10 g each. After sterilizing the pieces using 70% ethanol (v/v) **(figure 17)**, they were inoculated with 100 µL of a 10^{-6} dilution of a Salmonella strain isolated from the evisceration step sample **(figure 18),** achieving an initial concentration (N^0) of 7.8×10^{10} CFU/g. For each of the predetermined conditions (various temperatures and times), contaminated pieces were prepared to later count the bacterial cells post-incubation, thereby determining the effect of these factors on bacterial growth. Using the same method, we contaminated additional chicken fillet pieces with a second Salmonella strain isolated from the slaughtering step, with an initial concentration (N^0) of 4.3×10^9 CFU/g, and a reference strain of E. coli ATCC 25922, with an initial concentration (N^0) of 9.6×10^9 CFU/g.

Figure 16: sterilizing the chicken pieces using ethanol (70% v/v)

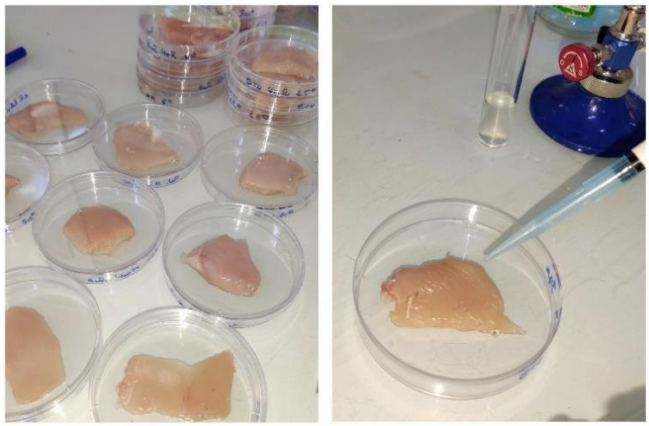


Figure 17: inoculation of the chicken fillets with 100 µL of the suspension

5.2. Microbial evaluation of bacteria inoculated in chicken fillets

To conduct the microbial analysis, chicken fillet samples weighing 10 g were blended for 5 min with sterile peptone water (90 m). Then, (10^{-1} to 10^{-3}) serial dilutions were made, 100 µL of 10^{-3} dilution were spread on pre-prepared nutrient agar media and the incubation occurred at 37°C. After the necessary incubation period which is 24h, the colonies present in each Petri dish were counted to finally conclude the number of bacterial cells that had grown:

$$[N] = \frac{\sum c}{(n1 + 0.1n2)d.V}$$

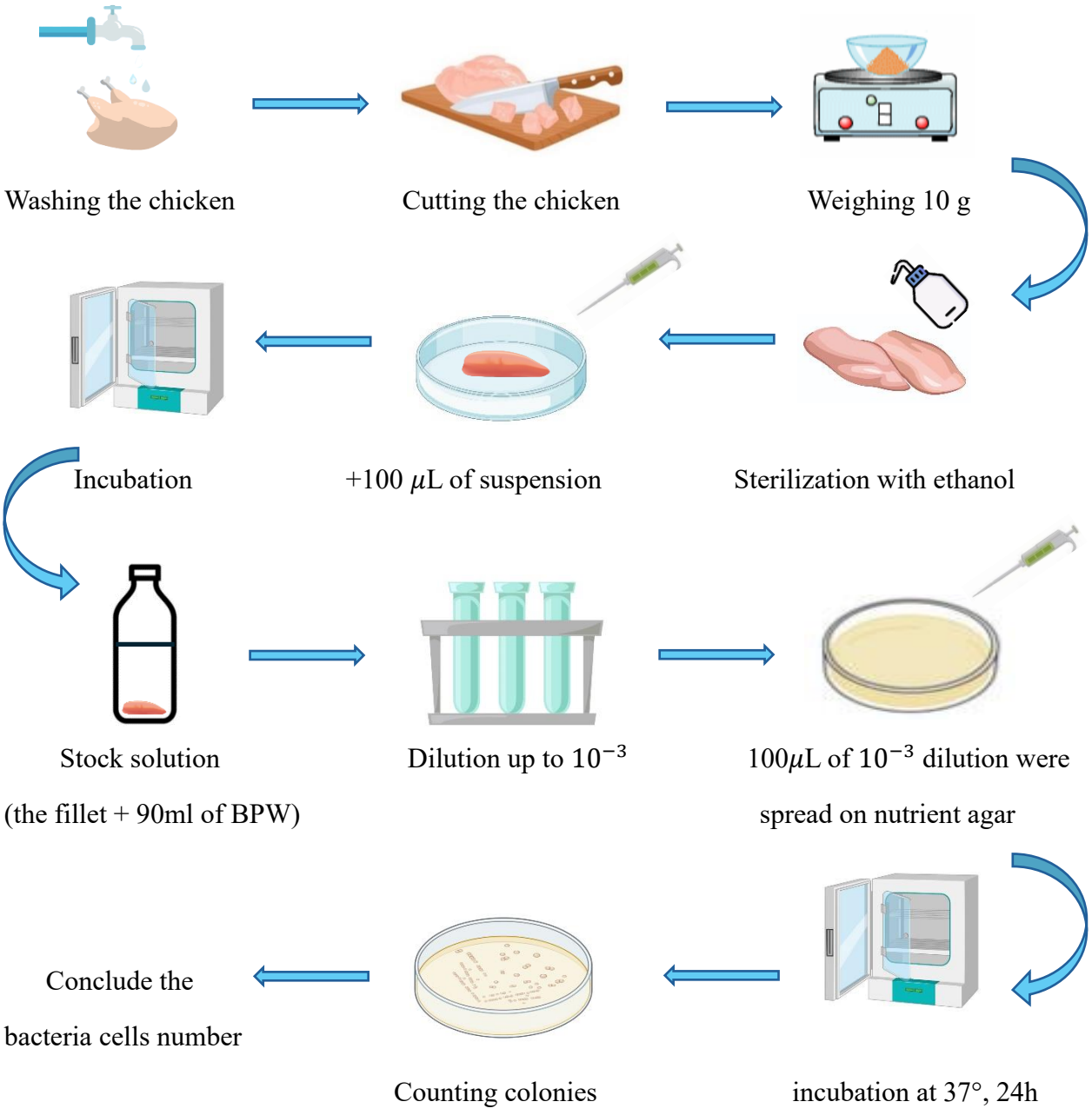


Figure 18: Diagram representing the contamination steps

6. Bacterial Growth kinetics modeling

The aim of predictive microbiology is to predict the evolution of microorganisms in food using mathematical models. Many primary and secondary models have been published; they are used to model the behavior of microorganisms (Delhalle et al 2012).

6.1. Primary models

They aim to reproduce the evolution of the concentration of microorganisms over time in a given environment.

The primary models used in our study are:

6.1.1. The logistic model with delay and disruption

It assumes the absence of growth during the latency and transition phase between this phase and the growth phase exponential (break). The model takes the following expression: (M. Sanaa1, 2002)

$$\log(N_t)=\log\left(\frac{N_{max}}{1+\left[\frac{N_{max}}{N_0}-1\right]\times\exp\left(-\mu_{max}\times time\right)}\right)$$

6.1.2. The Baranyi model

The Baranyi model is a kinetics of the passage of cells from the Lag phase to growth phase exponential. Is widely used because it gives satisfactory results when fitting models to laboratory data. The success of this model can be partly explained by the development of DMFit, a free adjustment software based on the primary Baranyi model available on http://www.combase.cc/toolbox.html (Delhalle et al 2012)

$$\ln\left[N(_t)\right]=\ln\left(N_0\right)+\mu_{max}A(t)-\ln\left(\frac{\exp\left(\mu_{max}.\ A(t)\right)-1}{\frac{N_{max}}{N_0}}\right)$$

6.1.3. The Gompertz model

The Gompertz mortality model as a predictor of the maximum life span (T_{max}), in terms of population size. (Delhalle et al 2012)

$$\ln\left(N(_t)\right)=\ln\left(N_0\right)+A\exp\left[-\exp\left[\frac{\mu e}{A}(lag-t)+1\right]\right]$$

6.2. Secondary modeling

Primary models, which describe microbial growth kinetics, often lack analytical solutions. Therefore, they must be integrated with secondary models. According to Li et al. (2017), secondary modeling involves quantifying the impact of environmental factors such as temperature, pH, and inhibitor concentrations on growth parameters like the maximum specific growth rate (μmax) and the lag phase duration. This approach links the primary growth models to the environmental conditions, providing a comprehensive understanding of microbial behavior under various conditions (Delignette-Muller, 1998; Baranyi et al., 1996).

The secondary models incorporated in our study to provide a more comprehensive and nuanced understanding of microbial behavior under different conditions, are:

6.2.1. Square root model

Square root model allows to improve the quality of adjustment by stabilizing the variance. The simplest model is that of Ratkowsky et al (1983). It allows you to evaluate the effect of temperature (M. sanaa, 2002)

$$\sqrt{\mu_{max}} = b \times (T - T_{min})$$

6.2.2. Cardinal model

Cardinal models are increasingly used empirical models (Le Marc et al., 2002; Van Derlinden et al., 2008). This model is based on the same principle of use as the gamma model, namely that the Influence of each environmental effect can be integrated into a function by multiplicative factors:

$$y(t) = \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt})(T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]}$$

6.2.3. The gamma concept

It was introduced by Zwietering et al. (1992) and is based on two principles:

- All measurable factors, having an influence on the growth rate (μ), are independent and intervene multiplicatively:
- The effect of each factor on growth rate can be represented by a fraction of the maximum growth rate:

$$\gamma = \frac{\mu}{\mu_{opt}} \quad \text{between 1 and 0}$$

$$\mu = f(temperature) \times f(a_w) \times f(ph) \times f(others_1) \times f(others_2) \times \ldots \times (others_n)$$

Square root type models can also be used in the gamma concept. Optimal growth rate (μ_{opt}) is the rate where all environmental conditions are optimal such as temperature (T_{opt}), pH (pH_{opt}), water activity (Aw_{opt}), etc. The combined effect of several environmental factors is then determined by multiplication of their respective gamma factors (Zwietering et al., 1996)

7. Statistical analysis and data fitting

We used different kinetic parameters to evaluate every model performance. So that in the end, we can choose which model represents the bacterial growth accurately. The residual sum of squares (RSS) were calculated. Then the mean square error (MSE) and root mean square error (RMSE) were calculated from RSS. In general, smaller RMSE indicate a better fit of the data (PinaPérez et al., 2009). Additionally, there are three other important metrics that were used: AIC, AICc and BIC that are commonly used for model evaluation and selection. These are an unbiased estimate of the model prediction error MSE. The lower these metrics, he better the model. Also, the analysis test of variance (ANOVA) and Tukey's test were used to get the best predictive model.

All Salmonella and *E.coli* populations were transformed to a base a 10 logarithm before analysis. Then the models were fitted to growth data by means of DMFit, a Microsoft Excel 2016, Combase and Graph Pad Prism 8.0.2 from each growth curve, three growth kinetic parameters were estimated, lag time (h), maximum growth rate μ_{max} (log CFU/h) and maximum population density MPD (log CFU/h). The significance of differences among values of each sample was determined by analysis of variance (ANOVA) and Tukey's test. Differences were considered significant at $p <$ 0:05 level.

Results and discussion

1. Microbiological analysis

1.1 Detection of Salmonella

In our investigation, microbiological analyses were conducted on chicken meat sourced from Laghouat city, with a specific focus on detecting Salmonella. Following culture incubation, we proceeded with colony characteristic identification. The findings revealed a 100% prevalence of Salmonella in 6 butcher shop samples as indicated in **Table 5**, where we employed 2 petri dishes per medium. In contrast, the prevalence in 21 slaughterhouse samples was determined to be 47.61% as depicted in **Table 6**.

Our methodology relied on macroscopic examination to derive these results. In the case of the SS medium, we considered colonies to be positive if they exhibited characteristics such as small or medium size, rounded shape, circular formation, smooth texture, and an uncolored appearance, indicating a lack of lactose fermentation. Additionally, the presence of a black center, resulting from hydrogen sulfide (H2S) production in the presence of thiosulfate and ferric citrate, also indicated a positive result (**figure 22**) (Goo,v et al,1973). Regarding the Hektoen medium, positive colonies displayed traits such as small, medium, or large size, circular shape, rounded edges, smooth texture, opaque appearance, and a green coloration with a black center, suggesting non-fermentation of the included sugars and production of H2S (**figure 20**). Any petri dishes lacking these morphological characteristics were deemed to have negative results (Madigan Michael T, et al 2018).

Table 5: the table represent the presence (+) or absence (-) of Salmonella aspect in each butcher's shop sample (the prevalence of Salmonella presence was 83% in SS and in Hektoen too)

Samples	SS	Hektoen
Neck	-+	++
Wing	++	--
Wing	++	++
Neck	++	++
Wing	++	++
Wing	--	+-

Table 6: the table represent the presence or absence of salmonella aspect in each slaughterhouse sample (the prevalence of Salmonella presence was 33% in SS and in Hektoen 23%).

Samples	SS	Hektoen
Evisceration 1 (Ev 1)	-	-
Evisceration 2 (Ev 2)	-	-
Evisceration 3 (Ev 3)	-	-
*Evisceration 4 (Ev 4)	+	-
*Evisceration 5 (Ev 5)	-	+
*Carcasses after evisceration 1(Cs 1)	+	+
*Carcasses after evisceration 2 (Cs 2)	+	-
*Carcasses after evisceration 3 (Cs 3)	-	+
*Carcasses after evisceration 4 (Cs 4)	+	-
*Carcasses after evisceration 5 (Cs 5)	+	-
After scalding 1 (Ec 1)	-	-
*After scalding 2 (Ec 2)	+	+
After scalding 3 (Ec 3)	-	-
After scalding 4 (Ec 4)	-	-
After scalding 5 (Ec 5)	-	-
After slaughter 1 (Ab 1)	-	-
*After slaughter 2 (Ab 2)	-	+
After slaughter 3 (Ab 3)	-	-
After slaughter 4 (Ab 4)	-	-
After slaughter 5 (Ab 5)	-	-
*Scalding water (O)	+	+

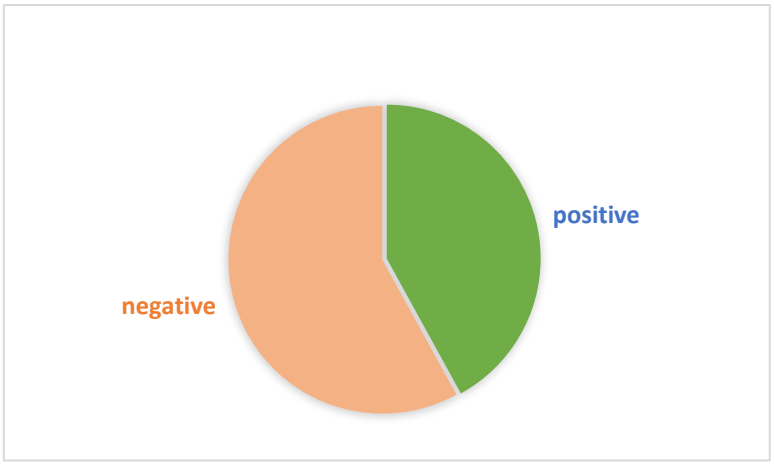


Figure 19: A relative circle representing the percentage of Salmonella in slaughterhouse samples.

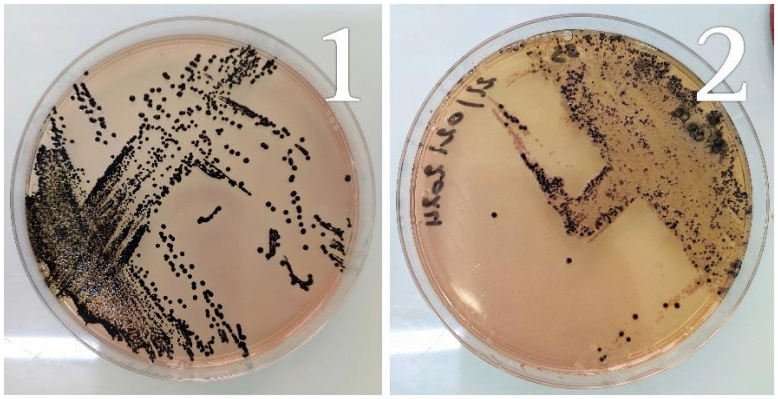


Figure 20: The appearance of Salmonella colonies on SS agar (1: O and 2: Ab 2).

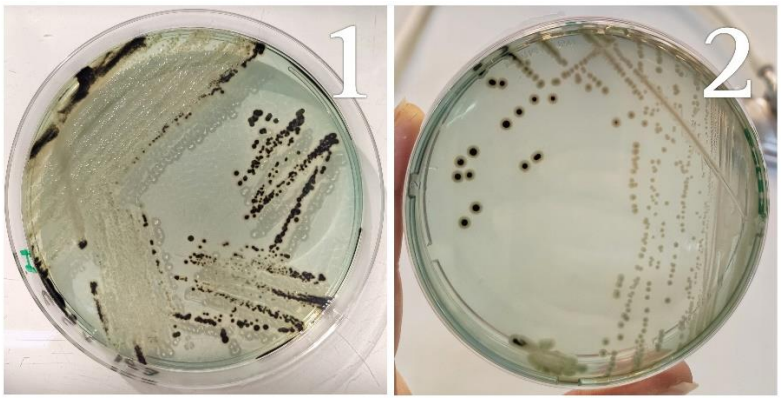


Figure 21: The appearance of Salmonella colonies on Hektoen agar (1: Ev 4 and 2: Cs 2).

2. Identification of salmonella

Following confirmation of the Salmonella presence in both butcher shop and slaughterhouse samples through macroscopic examination, we proceeded by selecting pure and well-isolated

colonies from the Petri dishes sourced from 10 slaughterhouse samples. These selected colonies underwent further identification through microscopic examination and biochemical assays.

2.1 Microscopic examination

2.1.1 Methylene blue staining

The microscopic identification of the isolates in the fresh state and after methylene blue staining allowed us to observed the bacteria in dark blue and in the form of mobile bacilli (**figure 23 and 24**).

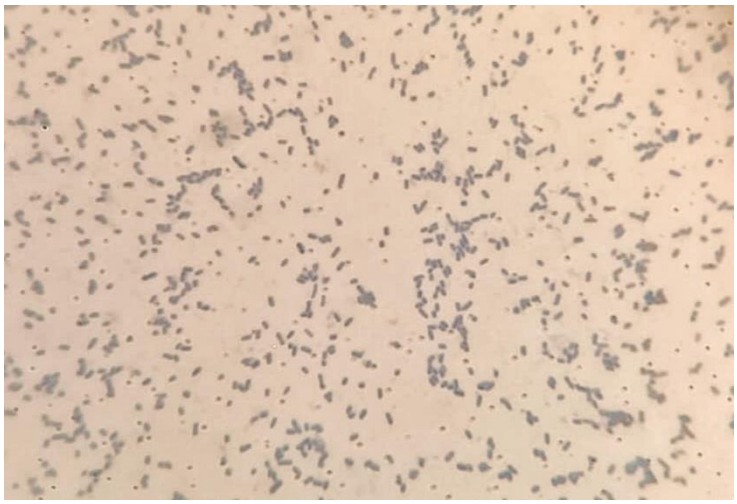


Figure 22: microscopic observation after Methylene blue staining (GR×100) of the stain isolated from the water scalding sample.

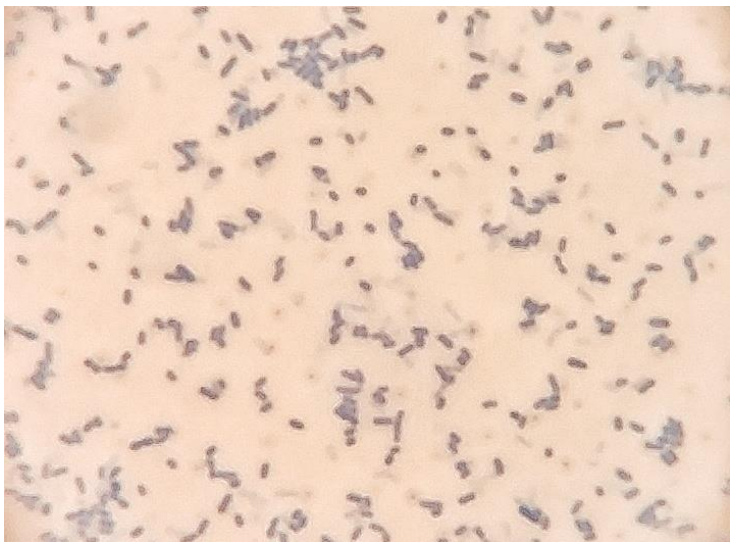


Figure 23: microscopic observation after Methylene blue staining (GR×100) of the stain isolated from the after scalding sample.

2.1.2 Gram staining

After performing the Gram stain, we observed under the optic microscope of pink-colored bacilli grouped in clusters or isolated or in small oak lets. So, our Salmonella stains are Gram negative bacteria (**figure 25, 26**). In this study the colony characteristics of *Salmonella* spp., observed on different media were similar to the findings of other authors like Quinn et al.,2002,

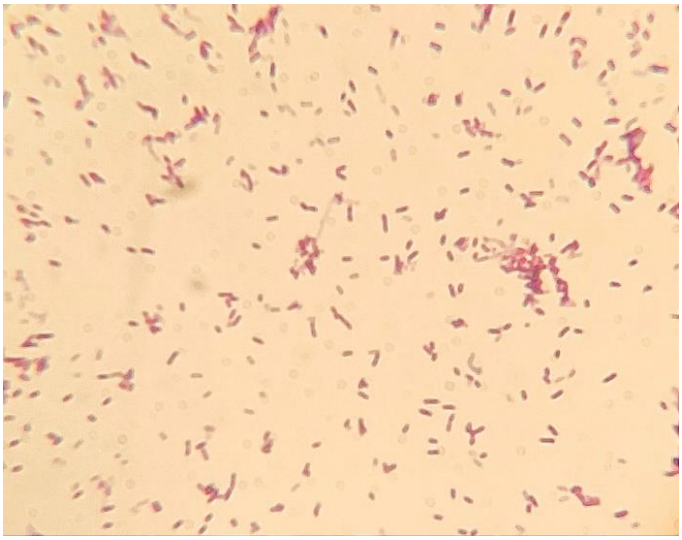


Figure 24: microscopic observation after Gram stain (GR×100) of the stain isolated from the water scalding sample.

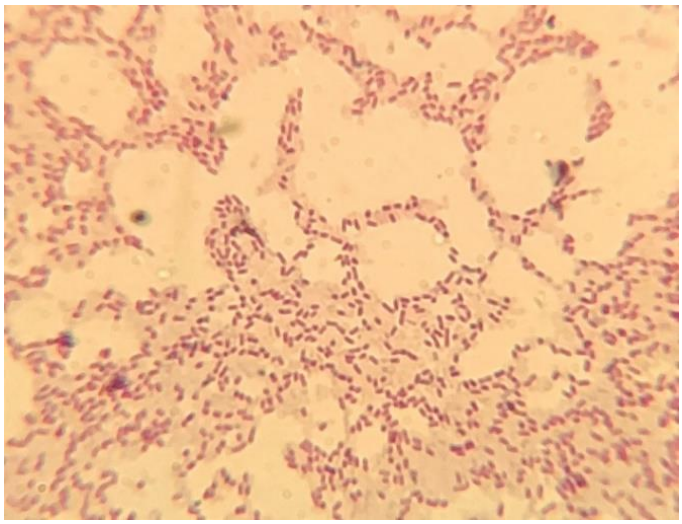


Figure 25: microscopic observation after Gram stain (GR×100) of the stain isolated from the after scalding sample.

2.2 Biochemical identification

2.2.1 Mannitol-motility test

The motility test serves as a foundational method for discerning motile and non-motile strains of Salmonella. Non-motile organisms typically correspond to either *S. pullorum* or *S. gallinarum*.

Conversely, motile organisms are identified as other species of Salmonella (OIE, 2004; Calnek et al., 1997; Chauhan and Roy, 1996).

The observed bacterial growth in all test tubes results in a phenol red color change to yellow, indicative of pH alteration (acidification of the medium). Thus, confirming mannitol fermentation in all strains (mannitol +). Motility is demonstrated by the formation of a veil around the central inoculation point, with all strains observed to be motile (**figure 27**). These combined characteristics strongly suggest that all tested strains are Salmonella, except for the two species, *S. pullorum* and *S. gallinarum.*

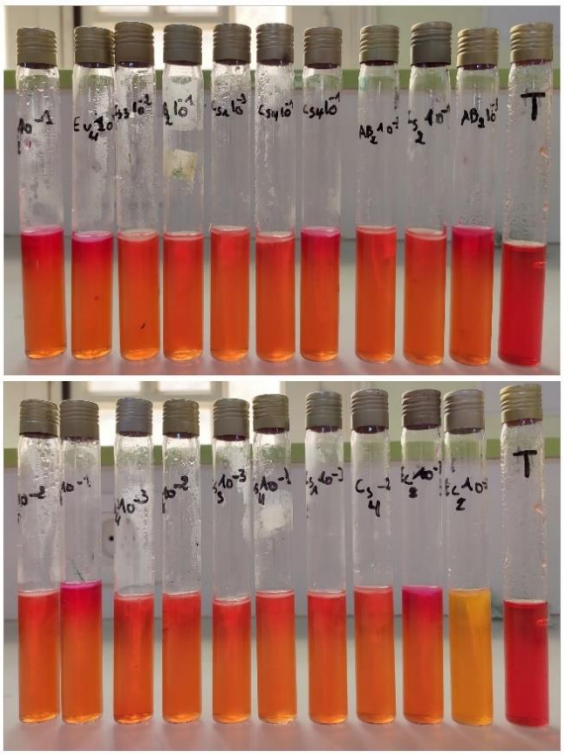


Figure 26: the results of mannitol-motility test.

2.2.2 Triple sugar Iron fermentation test

Positive results, indicating the presence of Salmonella, were determined by the following characteristics (**figure 27**): an alkaline (red) slant, suggesting that lactose and/or sucrose were not fermented, accompanied by an acidic (yellow) butt, indicating glucose fermentation. Additionally, the presence of bubbles or cracks signifies gas formation from glucose, while black precipitates indicate hydrogen sulfide (H2S) formation.

Conversely, tubes displaying an acidic (yellow) slant and an acidic (yellow) butt indicate fermentation of all sugars (glucose, lactose, and/or sucrose) and were considered negative results,

indicating the absence of Salmonella. This interpretation aligns with the criteria outlined by Buxton and Fraser (1977) (**Table 7**).

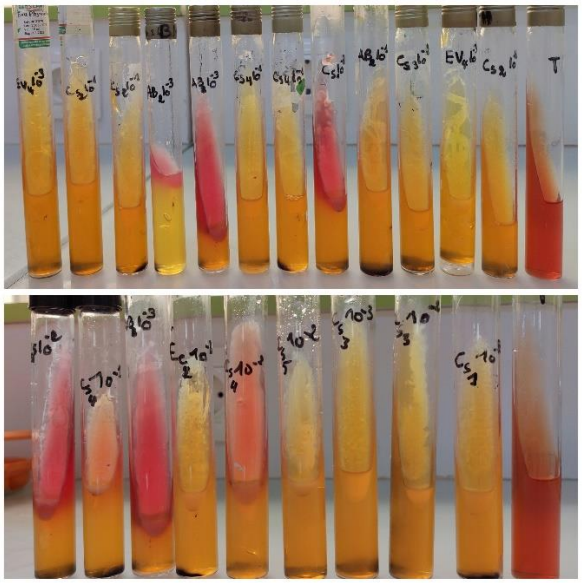


Figure 27: the results of Triple sugar Iron fermentation test.

Table 7: observation and interpretation of TSI results.

sample	slant	base	fermentation	gas production	Black base precipitation	Salmonella presence
Ev 4	yellow	yellow	All sugars	+	+	-
Cs 2	yellow	yellow	All sugars	+	+	-
Ab 2	red	yellow	glucose	+	+	+
Cs 1	red	yellow	glucose	-	+	+
Cs 3	yellow	yellow	All sugars	+	+	-
Cs 5	red	yellow	glucose	+	+	+
O	red	yellow	glucose	+	+	+
Cs 4	red	yellow	glucose	+	+	+
Ec 2	yellow	yellow	All sugars	-	+	-
Ev5	red	yellow	glucose	+	+	+

2.2.3 API ID 32 E plates

To confirm that the 10 isolates belonged to the Salmonella genus, identification was carried out using the API ID 32 E gallery, which is more discriminating than traditional biochemical tests. The tests revealed that all the stains belong the genus Salmonella spp. The isolation rate is

therefore 100% (10/10), two of them were chosen to be used in the contamination step which are Ev 5 and Ab 2.

The microscopic characteristics, gram reaction results, and biochemical tests, including the API ID 32 E test and glucose fermentation, collectively confirmed the isolated isolates as Salmonella spp. These species exhibit the unique ability to ferment monosaccharides while being unable to ferment disaccharides, a characteristic consistent with findings from several researchers (Tiabaijuka et al., 2003; Zschock et al., 2000; Merchant and Packer, 1967)

According to FAO (1997), Salmonella represents a bacterium with significant pathogenic potential. Once detected in food, the latter is deemed unsafe for human consumption. In our study, we discovered that 47.61% (10/21) of the slaughterhouse samples contained Salmonella. This finding is in line with results from studies conducted by Ahmed et al. (2007), Habib-Ur-Rehman et al. (2004), and Biswas et al. (2004), which investigated Salmonella spp. prevalence under similar environmental conditions in India and Bangladesh. Conversely, our overall isolation rate exceeds that reported in Iraq (4%) (Nader et al., 2015) and Egypt (14%) (Rabie et al., 2012). Notably, at the slaughterhouse level, we observed higher proportions of Salmonella in the intestines, a trend consistent with findings reported in Algeria by El Groud et al. (2008).

3. The study of the growth of E. coli and two stains of Salmonella in chicken meat

The table below displays the temperature and pH measurements for each sample collected from butcher's shops (**Table 8,9,10**), slaughterhouses, and home-stored chicken meat, as mentioned in the sample collection step. From the collected data, we identified four critical temperatures closest to the cardinal temperatures. These temperatures were selected meticulously to ensure that our results closely approximate reality.

Table 8: The temperatures and pH samples measured in the butcher's shop.

sample	Surface T°c	Depth T°c	pH
Wing 1	16.1	13.3	7.1
Wing 2	12.8	13.5	7
Neck	13.9	12.9	6.9
Wing 1	13.2	12.9	6.7
Wing 2	12.9	12.4	6.9
Neck	13	13	7

Table 9: The temperature and pH samples measured in the slaughterhouse.

sample	T°c	pH
Slaughter	36	7.5
Scalding water	41.7	7.96
After scalding 1	24	7.74
After scalding 2	19.3	7.57
After scalding 3	18.3	6.4
After scalding 4	20.1	6.69
After scalding 5	20.8	7.18
Evisceration	20.8	7.2
carcasses evisceration	19.9	7.13

Table 10: the means of butcher and fridge measured temperatures.

Mean T° of Butcher	Mean T° of Fridge
13,1266289∓3,88041108	10,3333385∓1,37093958
14,5903259∓3,01562324	5,78455261∓0,74147753
14,1514302∓3,31402691	8,06870464∓2,40667375

Following the experiment and the enumeration of bacterial colonies, the number of bacterial cells was determined using the ISO 7218 AFNOR standard equation.

$$[N]\frac{\sum c}{(n_1 + 0.1n_2)d.V}$$

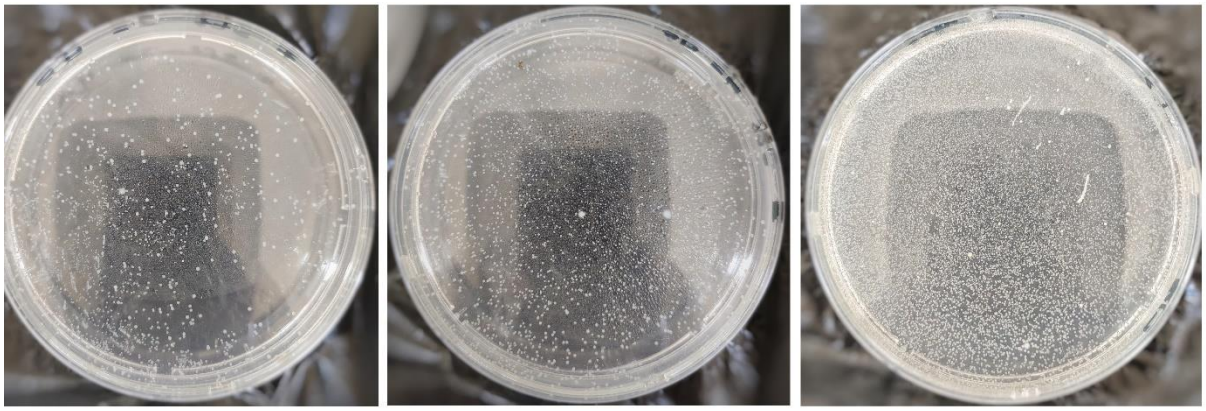


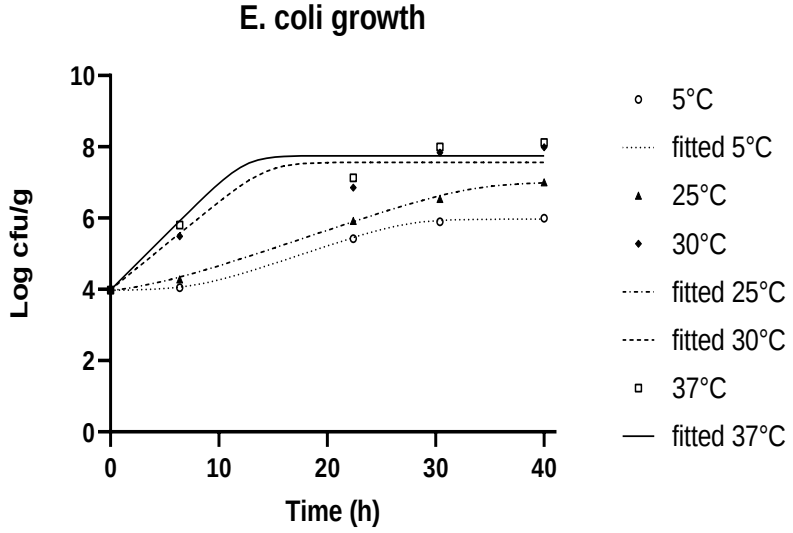
Figure 28: A few petri dishes of counting.

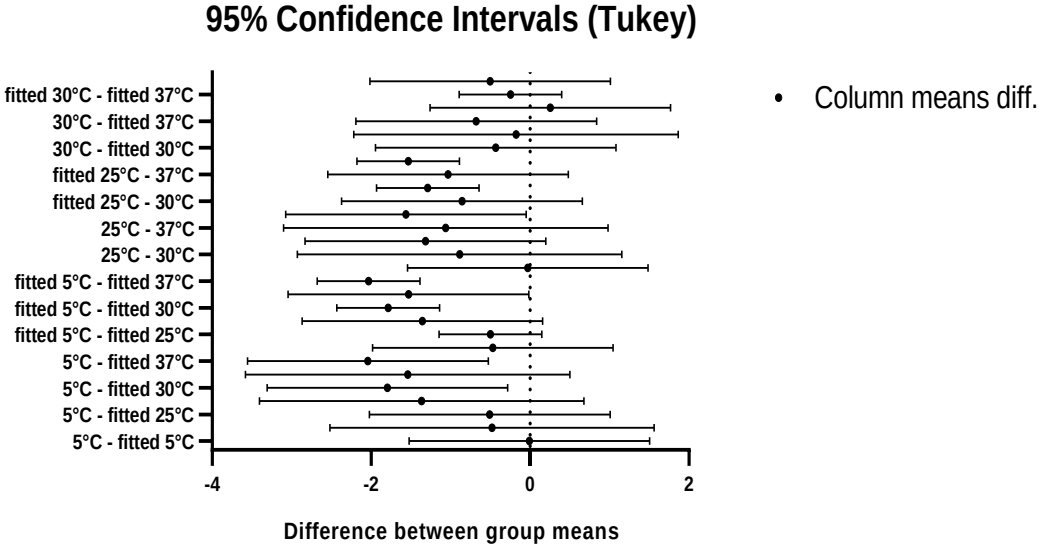
3.1 Primary modeling

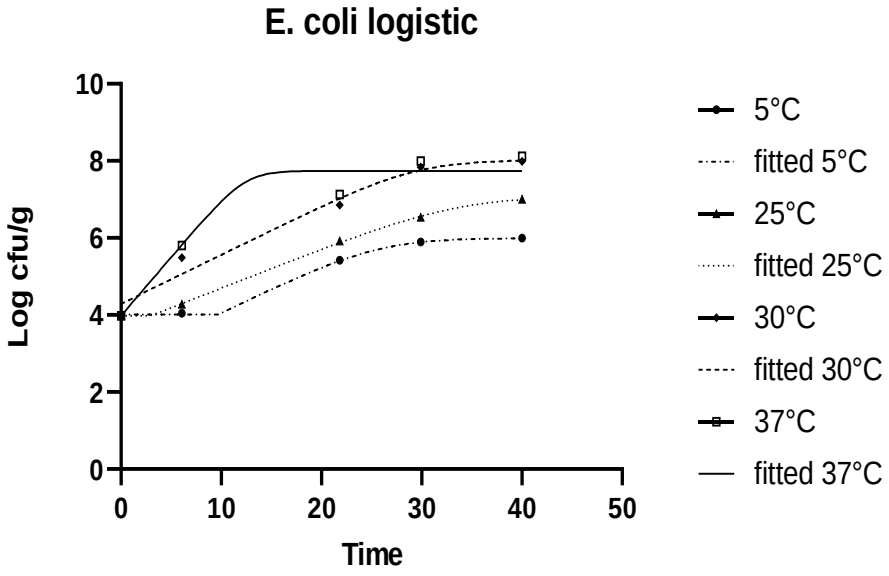
3.1.1 E.coli ATCC 25922

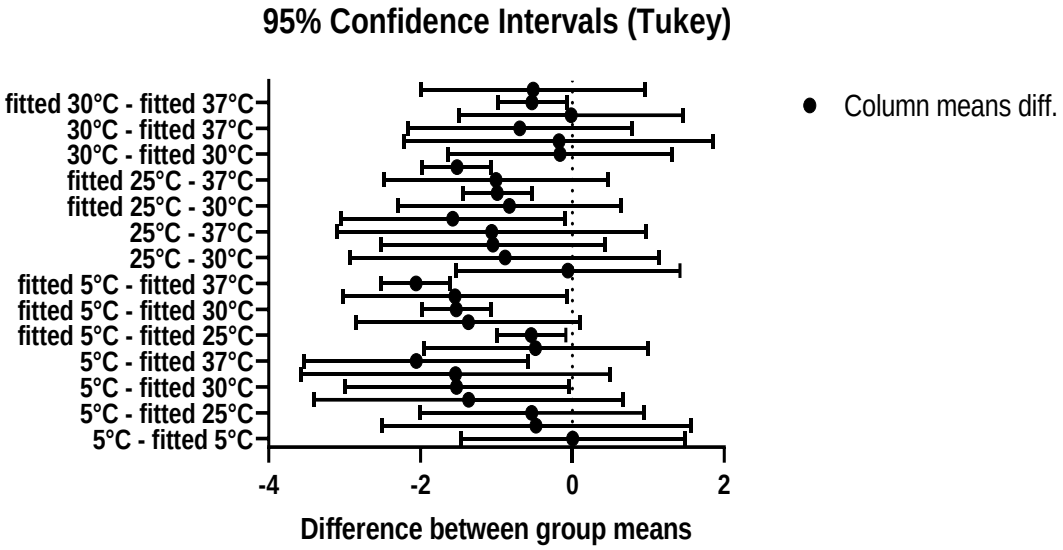
Table 11: counting *E.coli* cells results LOG UFC/g.

Time(h) / *T°C*	05ºC	25ºC	30ºC	37ºC
00h	3,98	3,98	3,98	3,98
06h	4,04	4,27	5,48	5,80
22h	5,42	5,92	6,85	7,12
30h	5,89	6,53	7,84	7,99
40h	5,99	7,00	7,98	8,12






E. coli logistic
Log cfu/g
Time
0
2
4
6
8
10
0
10
20
30
40
50
5°C
fitted 5°C
25°C
fitted 25°C
30°C
fitted 30°C
37°C
fitted 37°C


95% Confidence Intervals (Tukey)
fitted 30°C - fitted 37°C
30°C - fitted 37°C
30°C - fitted 30°C
fitted 25°C - 37°C
fitted 25°C - 30°C
25°C - 37°C
25°C - 30°C
fitted 5°C - fitted 37°C
fitted 5°C - fitted 30°C
fitted 5°C - fitted 25°C
5°C - fitted 37°C
5°C - fitted 30°C
5°C - fitted 25°C
5°C - fitted 5°C
-4
-2
0
2
Difference between group means
Column means diff.

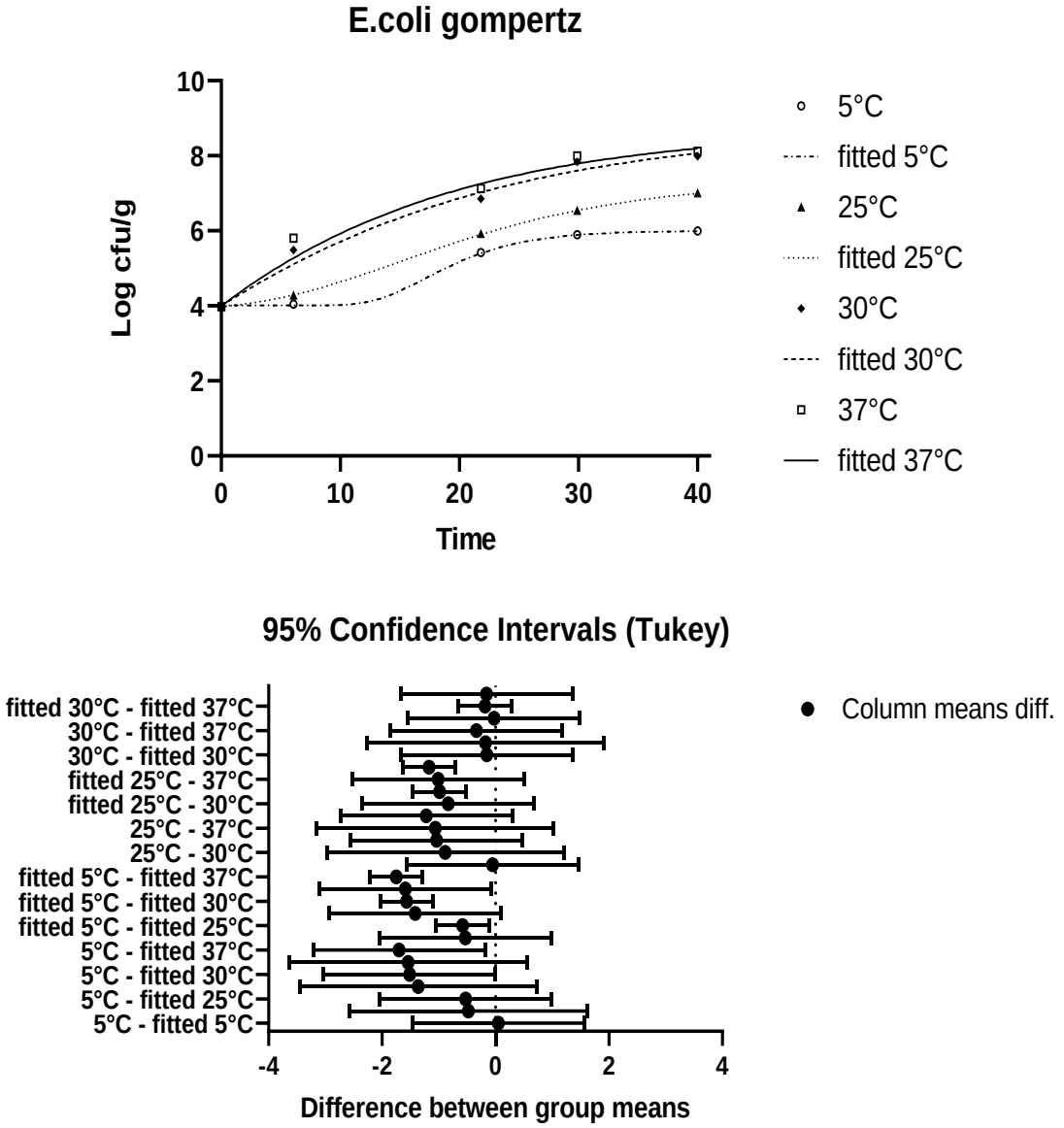


Figure 29: growth of *E.coli* in chicken meat at various temperatures described with Baranyi, Logistic and Gompertz models with their Tukey's test results.

The results of the statistical analysis (ANOVA) for the growth of *E.coli* strain that was adjusted by the Baranyi model, at different temperatures, the p value ($p= 0.4238 > 0.05$). And for the Logistic model, the p value was ($p=0,0008 < 0.05$). But on the other hand the Gompertz model has a p value ($p= 0.1201 > 0.05$). After conducting the ANOVA test, we still need to understand the differences between the different growth temperatures. To achieve this, we used the Tukey's test, the results of which are shown in the (**figure 30**), where we noticed in all the models that there is no noticeable difference in growth of temperatures 5°C and 25°C, but as for the temperatures 5°C and 30°C there is a very prominent difference compared to the above. The same goes for 5°C and

37°C, as we noticed almost the same difference between them. This is due to the fact that 30°C and 37°C do not have a significant difference between them, as shown in the figure. As for growth at the two temperatures 25°C with 30°C and 37°C shows a significant difference, but not a very large one.

Escherichia coli, being a typical mesophile, can sustain balanced growth within a temperature range spanning from 10 to nearly 49°C, the upper limit being contingent upon the available nutrients in the medium. Within this temperature spectrum, particularly between approximately 20 to 37°C, the growth rate of *E. coli* varies with temperature, generally increasing with higher temperatures (Sommers et al., 2018). However, growth significantly slows above 40°C or below 20°C, ultimately ceasing entirely at 9 or 49°C (Herendeen, 1979).

Studies have shown that *E. coli* does not proliferate in chicken meat at 5°C (Sommers et al., 2018), a common refrigeration temperature being 4°C. Additionally, it did not demonstrate growth in hydroponic lettuce at the same temperature. G.D. Betts (2000) discovered that *E. coli* could grow down to a minimum temperature of 6.5°C, albeit in media differing by 1-2°C compared to foods like raw beef and ground chicken in various media.

However, our results contradict these findings as we observed normal growth at temperatures of 25°C, 30°C, and 37°C. This discrepancy might be attributed to the mesophilic nature of this bacteria. Yet, at 5°C, we observed abnormal growth compared to previous studies. This unexpected growth could stem from chicken components fostering E. coli growth, or it may be attributed to potential technical errors during the experiment, particularly during the incubation step.

Table 12: Comparison of statistics obtained from three primary models of *E.coli*. the goodness-of-fit was estimated by calculating the RSS, MSE, RMSE, AIC, BIC.

T°c	Model	RSS	MSE	RMSE	AIC	BIC
5°c	Baranyi	0	0	0.17	-18.145	-19.707
	Logistic	0.002	0.002	0.042	-9.117	-10.68
	Gompertz	0.002	0.002	0.042	-9.119	-10.681
25°c	Baranyi	0.06	0.06	0.075	-3.378	-4.941
	Logistic	0.002	0.002	0.045	-8.6	-10.162
	Gompertz	0	0	0.009	-24.189	-25.752
30°c	Baranyi	0.072	0.072	0.268	9.297	7.734
	Logistic	0.327	0.163	0.404	14.888	13.716
	Gompertz	0.098	0.098	0.314	10.883	9.321
37°c	Baranyi	0.053	0.053	0.23	7.804	6.242
	Logistic	0.591	0.295	0.543	17.85	16.678
	Gompertz	0.069	0.069	0.262	9.08	7.518

The results illustrated in the (**table 12**): exhibit a slight difference between the observed values and the predictions of the Baranyi, Logistic, Gompertz, indicating that these models successfully describe the growth pattern of E. coli ATCC 25922 on the chicken meat. To compare the accuracy of the models, seven key parameters were analyzed for each model RMSE, RSS, MSE, AIC, AICc, BIC. Although the values for all three models, the Baranyi model exhibited the smallest AIC and RMSE, RSS, MSE, BIC parameters in 5°c, 30°c,37°c Consequently, the Baranyi model was identified as the most suitable growth model for *E. coli* in this experiment.

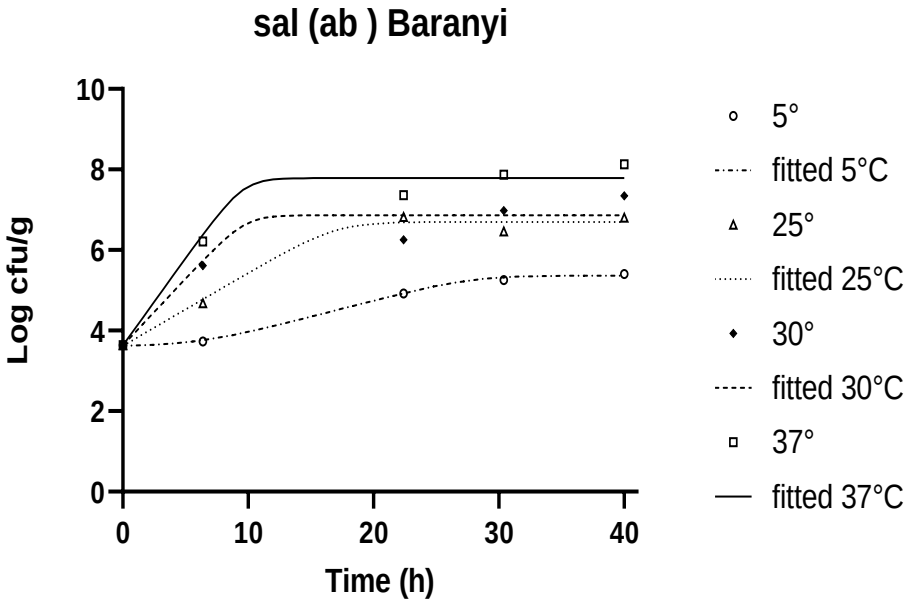
Table 13: Growth kinetics values of *E.coli* in chicken meat, calculated from the Baranyi and Logistic and Gompertz equations.

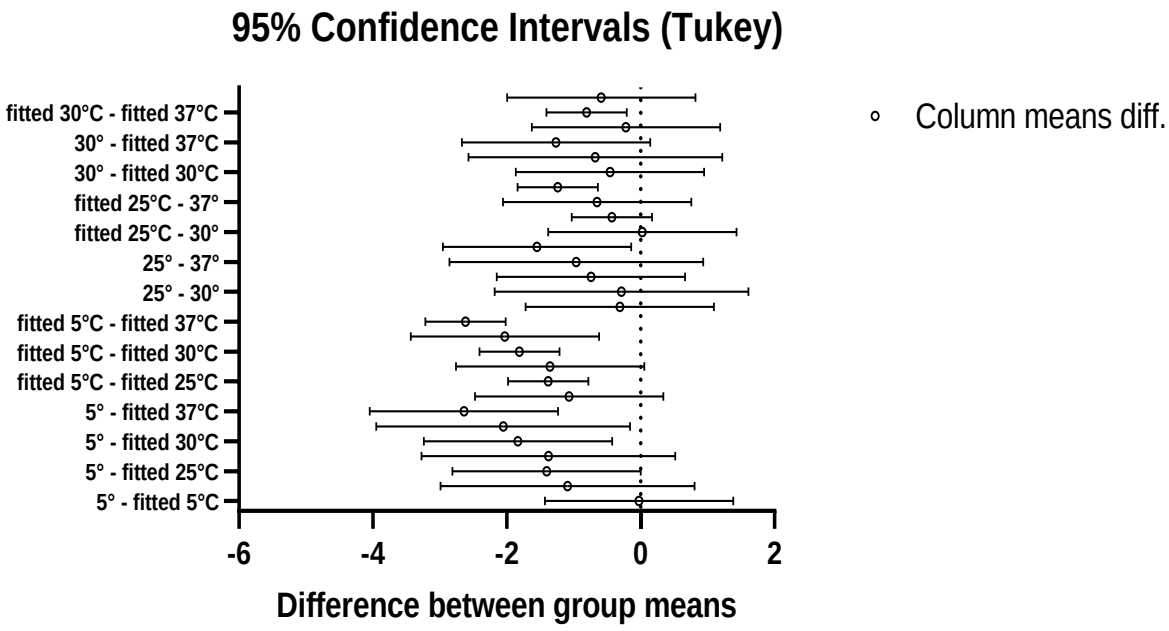
T°	Growth kinetics	Baranyi	Logistic	Gompertz
5°c	N_0	3.973 ± 0.0414	4,01	4,01
	μ_{max}	0.102 ± 0.0109	0,287	0,376
	Lag time	7.791 ± 1.76	9,66	12,746
	N_{max}	5.969 ± 0.0357	5,997	6,003
25°c	N_0	3.968 ± 0.0964	3,98	3,892
	μ_{max}	0.103 ± 0.00929	0,235	0,257
	Lag time	3.524 ± 2.221	2,966	3,512
	N_{max}	7.0208 ± 0.116	7,073	7,366
30°c	N_0	3.99 ± 0.613	4,287	-4,904
	μ_{max}	0.248 ± 0.145	0,294	0,61
	Lag time			-35,045
	N_{max}	7.563 ± 0.355	8,023	8,756
37°c	N_0	3.983 ± 0.543	3,982	365,122
	μ_{max}	0.304 ± 0.129	0,698	1,263
	Lag time			329,266
	N_{max}	7.748 ± 0.314	7,744	1,349

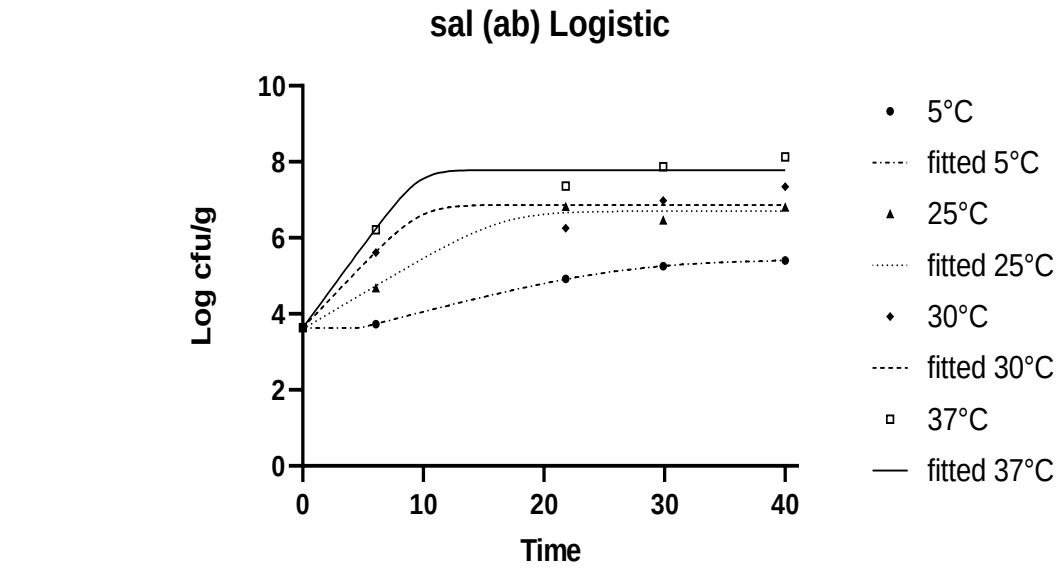
3.1.2 Salmonella (Ab 2)

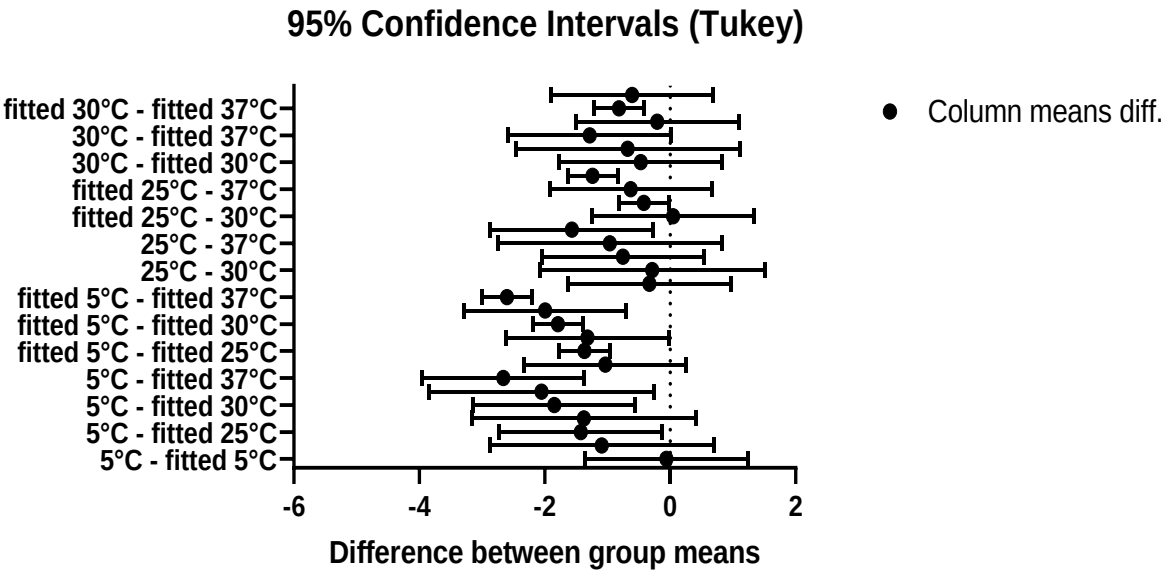
Table 14: counting Salmonella (Ab 2) cells results LOG UFC/g.

Time(h) / *T°C*	05ºC	25ºC	30ºC	37ºC
00h	3,633	3,633	3,633	3,633
06h	3,730	4,681	5,608	6,214
22h	4,918	6,819	6,255	7,362
30h	5,256	6,464	6,978	7,869
40h	5,404	6,801	7,349	8,125


sal (ab) Baranyi
Log cfu/g
Time (h)
0
2
4
6
8
10
0
10
20
30
40
5°
fitted 5°C
25°
fitted 25°C
30°
fitted 30°C
37°
fitted 37°C


95% Confidence Intervals (Tukey)
fitted 30°C - fitted 37°C
30° - fitted 37°C
30° - fitted 30°C
fitted 25°C - 37°
fitted 25°C - 30°
25° - 37°
25° - 30°
fitted 5°C - fitted 37°C
fitted 5°C - fitted 30°C
fitted 5°C - fitted 25°C
5° - fitted 37°C
5° - fitted 30°C
5° - fitted 25°C
5° - fitted 5°C
-6
-4
-2
0
2
Difference between group means
Column means diff.


sal (ab) Logistic
Log cfu/g
Time
0
2
4
6
8
10
0
10
20
30
40
5°C
fitted 5°C
25°C
fitted 25°C
30°C
fitted 30°C
37°C
fitted 37°C


95% Confidence Intervals (Tukey)
fitted 30°C - fitted 37°C
30°C - fitted 37°C
30°C - fitted 30°C
fitted 25°C - 37°C
fitted 25°C - 30°C
25°C - 37°C
25°C - 30°C
fitted 5°C - fitted 37°C
fitted 5°C - fitted 30°C
fitted 5°C - fitted 25°C
5°C - fitted 37°C
5°C - fitted 30°C
5°C - fitted 25°C
5°C - fitted 5°C
-6
-4
-2
0
2
Difference between group means
Column means diff.

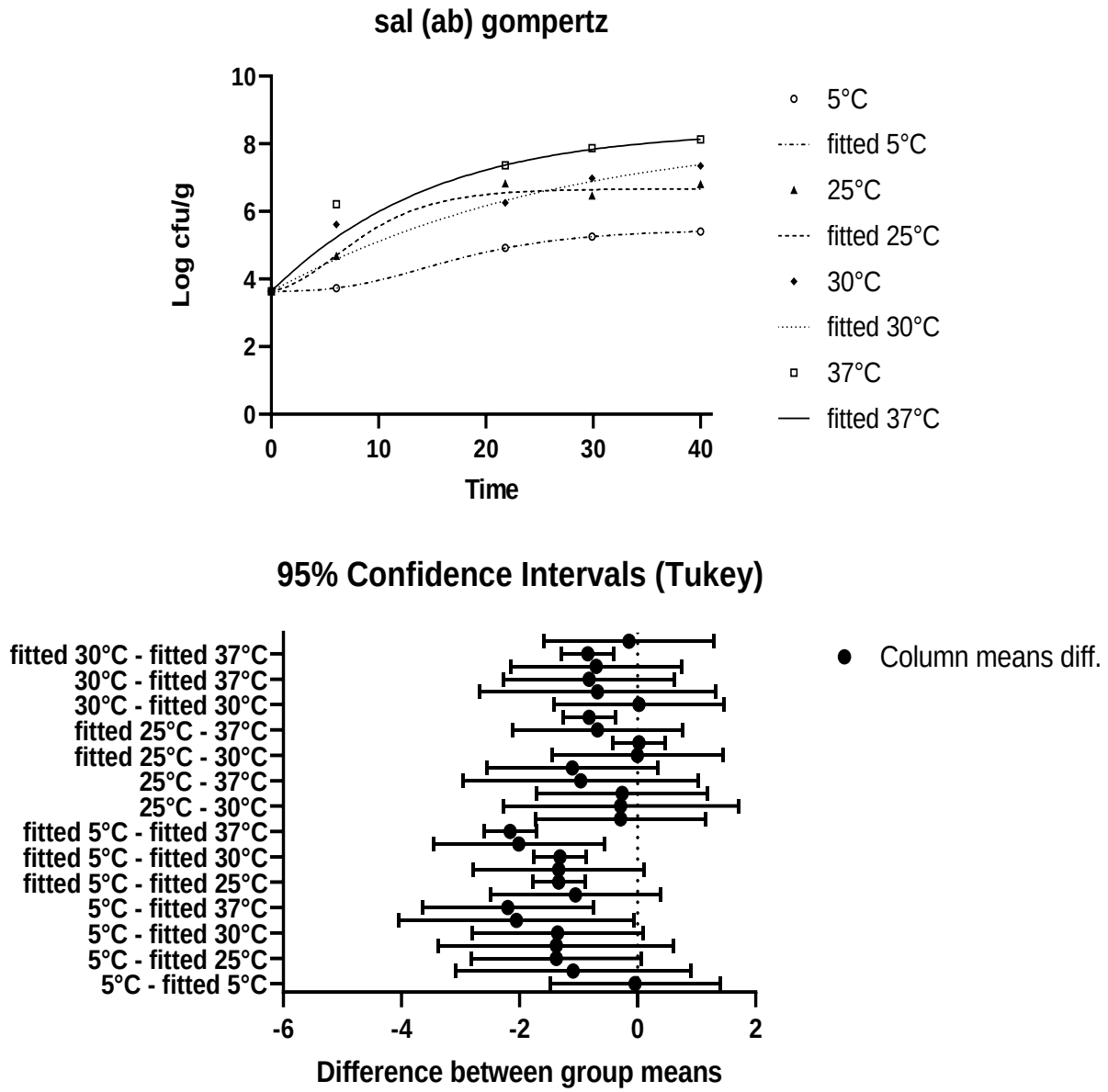


Figure 30: growth of Salmonella (AB2) in chicken meat at various temperatures described with Baranyi model, Logistic model, Gompertz model with their Tukey's test results.

According to the results of the statistical analysis (ANOVA) for the growth of Salmonella (AB 2) that was adjusted by the Baranyi model, at different temperatures, the p value (p= 0,4045> 0.05). for the Logistic model, the p value was (p=0,1551>0.05). But on the other hand the Gompertz model has a p value (p= 0,0006<0.05). In order to know the differences between the different growth temperatures, we used the Tukey's test, the results of which are shown in the (**figure 31),** where we noticed in all the models that there is no noticeable difference in growth of temperatures 5°C and 25°C, but for the temperatures 5°C and 30° we noticed a notable difference. The same goes for 5°C and 37°C there is a very prominent difference compared to the above. This is due to the fact that 30°C and 37°C have a significant difference between them.

And for the difference between the growth at the two temperatures 25°C and 37°C shows a small significant difference.

Salmonella, being a mesophilic bacterium, exhibits limited or inhibited growth in vitro at temperatures below 10°C and above 42°C, with its optimal growth temperature zone lying between 35 and 37°C (Bryan et al., 1979). Upon analyzing our counting results, we observed a notable increase in Salmonella population as temperatures increased and approached the optimal range. This finding is consistent with numerous studies represented in (**figure 31**).

Studies conducted on Salmonella growth at temperatures below 10°C have yielded varied results. Young-Jo Kim et al. (2018) and EFSA Panel on Biological Hazards (2014) reported that Salmonella cannot proliferate at 5°C. Similarly, Whiting et al. (1979), Gurtler and Conner (2009), and Singh et al. (2011) found that the minimum growth temperature for Salmonella ranged between 6-8°C. However, D'Aoust (1991) described that certain strains of Salmonella spp. have demonstrated the ability to grow at low temperatures, such as 5.9°C in complex broth, 2°C on fresh meat, and 4°C on agar and eggshells, as reported by Lanciotti et al. (2001), where 4.69°C was identified as the minimum growth temperature for Salmonella.

Our findings suggest that Salmonella can indeed grow at a temperature of 5°C, aligning with the latter set of studies. However, the rapid growth observed in our case, which contrasts with the results of many other studies described in (**table 14**), may indicate a technical issue during incubation, rendering our results unrealistic.

Table 15: Comparison of statistics obtained from three primary models of Sal (Ab 2) the goodness-of-fit was estimated by calculating the RSS, MSE, RMSE, AIC, BIC.

T°c	Model	RSS	MSE	RMSE	AIC	BIC
5°c	Baranyi	0.002	0.002	0.04	-9.619	-11.181
	Logistic	0	0	0	-72.122	-73.648
	Gompertz	0	0	0.008	-25.541	-27.103
25°c	Baranyi	0.71	0.355	0.596	18.77	17.598
	Logistic	0.095	0.047	0.218	8.704	7.53
	Gompertz	0.055	0.055	0.234	7.962	6.4
30°c	Baranyi	0.078	0.039	0.197	7.722	6.55
	Logistic	0.614	0.307	0.554	18.047	16.876
	Gompertz	0.015	0.015	0.121	1.402	-0.16
37°c	Baranyi	0.005	0.005	0.068	-4.395	-5.957
	Logistic	0.3	0.15	0.387	14.464	13.292
	Gompertz	0.002	0.002	0.043	-8.922	-10.448

The (**Table 15**) depicts a congruence between observed values and predictions from the Baranyi, Logistic, and Gompertz models, suggesting their efficacy in describing Salmonella (Ab 2) growth on chicken meat. The table compares RMSE, RSS, MSE, AIC, AICc, and BIC across the models. Notably, the Gompertz model shows the lowest values for AIC, RMSE, RSS, MSE, and BIC at 25°C, 30°C, and 37°C. Consequently, the Gompertz model emerges as the most suitable growth model for Salmonella (AB 2) in this experimental setup.

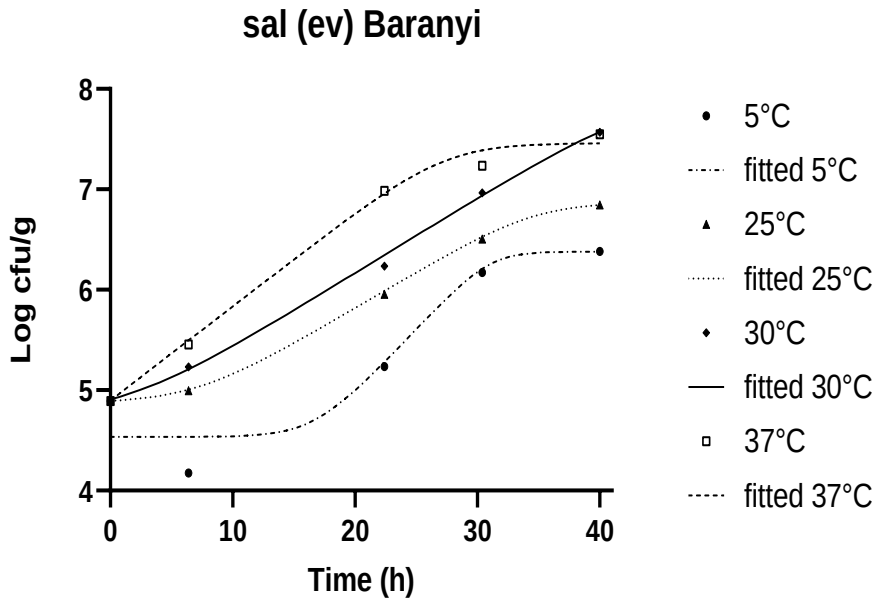
Table 16: Growth kinetics values of Sal (AB 2) in chicken meat, calculated from the Baranyi and Logistic and Gompertz equations.

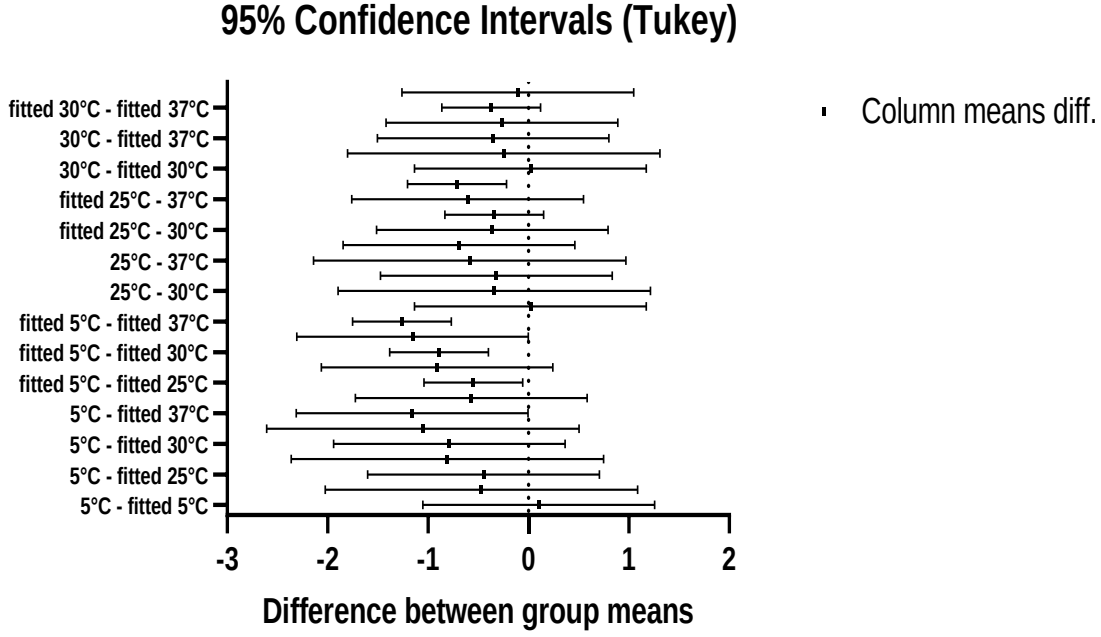
T°	Growth kinetics	Baranyi	Logistic	Gompertz
5°c	N_0	3.623 ± 0.0757	3,63	3,628
	μ_{max}	0.0809 ± 0.0124	0,193	0,206
	Lag time	6.13 ± 2.852	4,786	6,498
	N_{max}	5.365 ± 0.0662	5,429	5,461
25°c	N_0	3.618 ± 0.205	3,598	3,531
	μ_{max}	0.181 ± 0.0479	0,434	0,549
	Lag time			1,172
	N_{max}	6.697 ± 0.122	6,702	6,666
30°c	N_0	3.633 ± 0.557	3,63	-6,41
	μ_{max}	0.33 ± 0.133	0,769	0,553
	Lag time			-44,292
	N_{max}	6.861 ± 0.321	6,863	8,301
37°c	N_0	3.633 ± 0.388	3,63	-13,42
	μ_{max}	0.432 ± 0.0931	0,995	1,374
	Lag time			-32,276
	N_{max}	7.785 ± 0.224	7,783	8,409

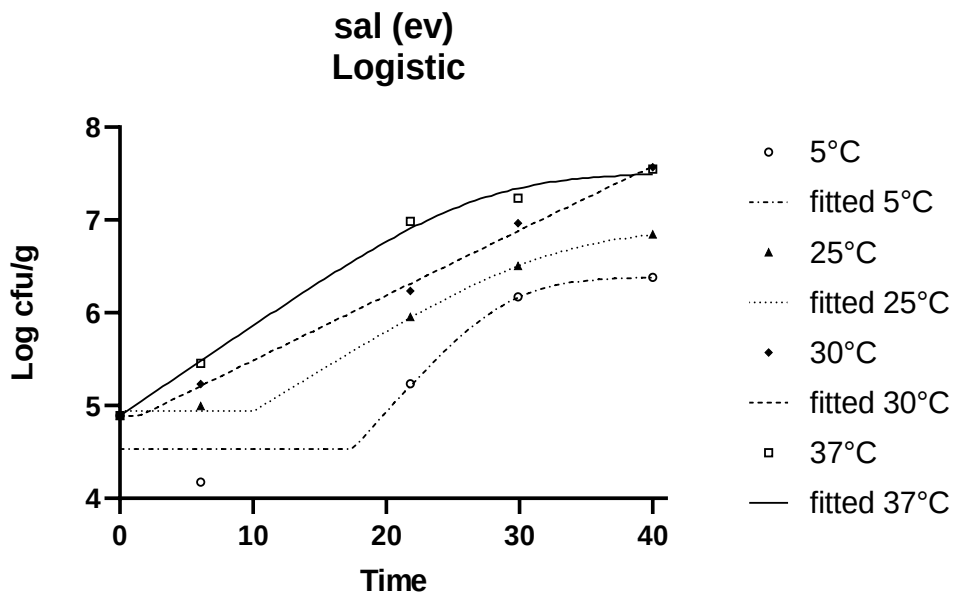
3.1.3 Salmonella (Ev 5)

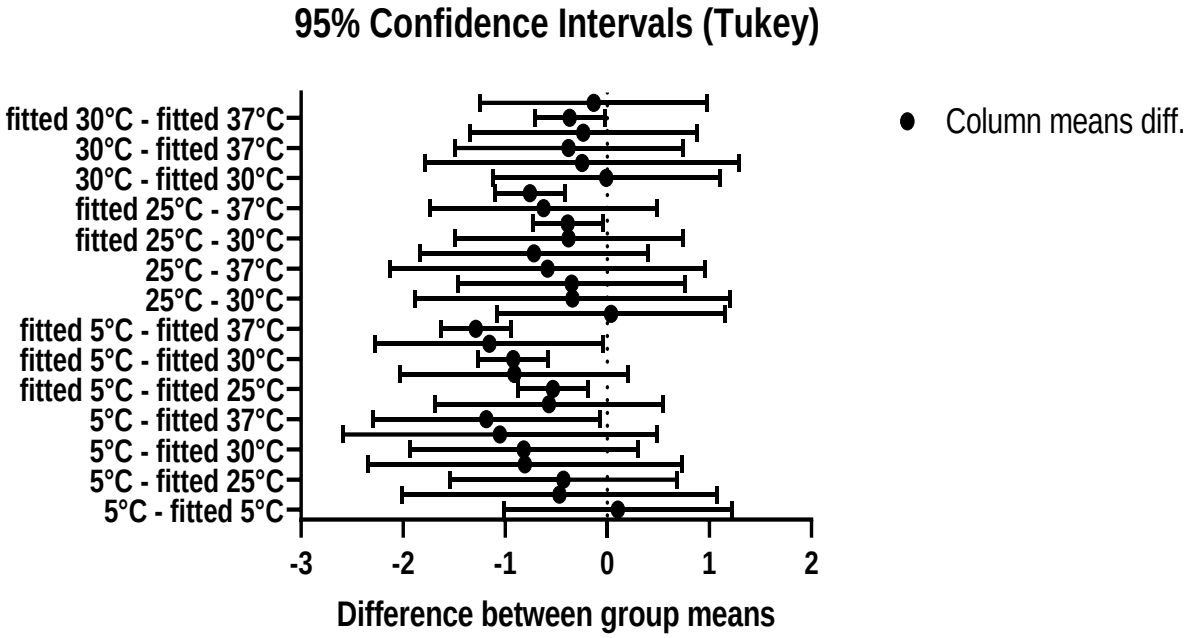
Table 17: counting Salmonella (Ev 5) cells results LOG UFC/g.

Time(h) / *T°C*	5ºC	25ºC	30ºC	37ºC
00h	4,892	4,892	4,892	4,892
06h	4,176	4,996	5,239	5,456
22h	5,236	5,956	6,236	6,984
30h	6,172	6,505	6,965	7,236
40h	6,380	6,844	7,569	7,548






sal (ev)
Logistic
Log cfu/g
Time
8
7
6
5
4
0
10
20
30
40
5°C
fitted 5°C
25°C
fitted 25°C
30°C
fitted 30°C
37°C
fitted 37°C


95% Confidence Intervals (Tukey)
fitted 30°C - fitted 37°C
30°C - fitted 37°C
30°C - fitted 30°C
fitted 25°C - 37°C
fitted 25°C - 30°C
25°C - 37°C
25°C - 30°C
fitted 5°C - fitted 37°C
fitted 5°C - fitted 30°C
fitted 5°C - fitted 25°C
5°C - fitted 37°C
5°C - fitted 30°C
5°C - fitted 25°C
5°C - fitted 5°C
-3
-2
-1
0
1
2
Difference between group means
Column means diff.

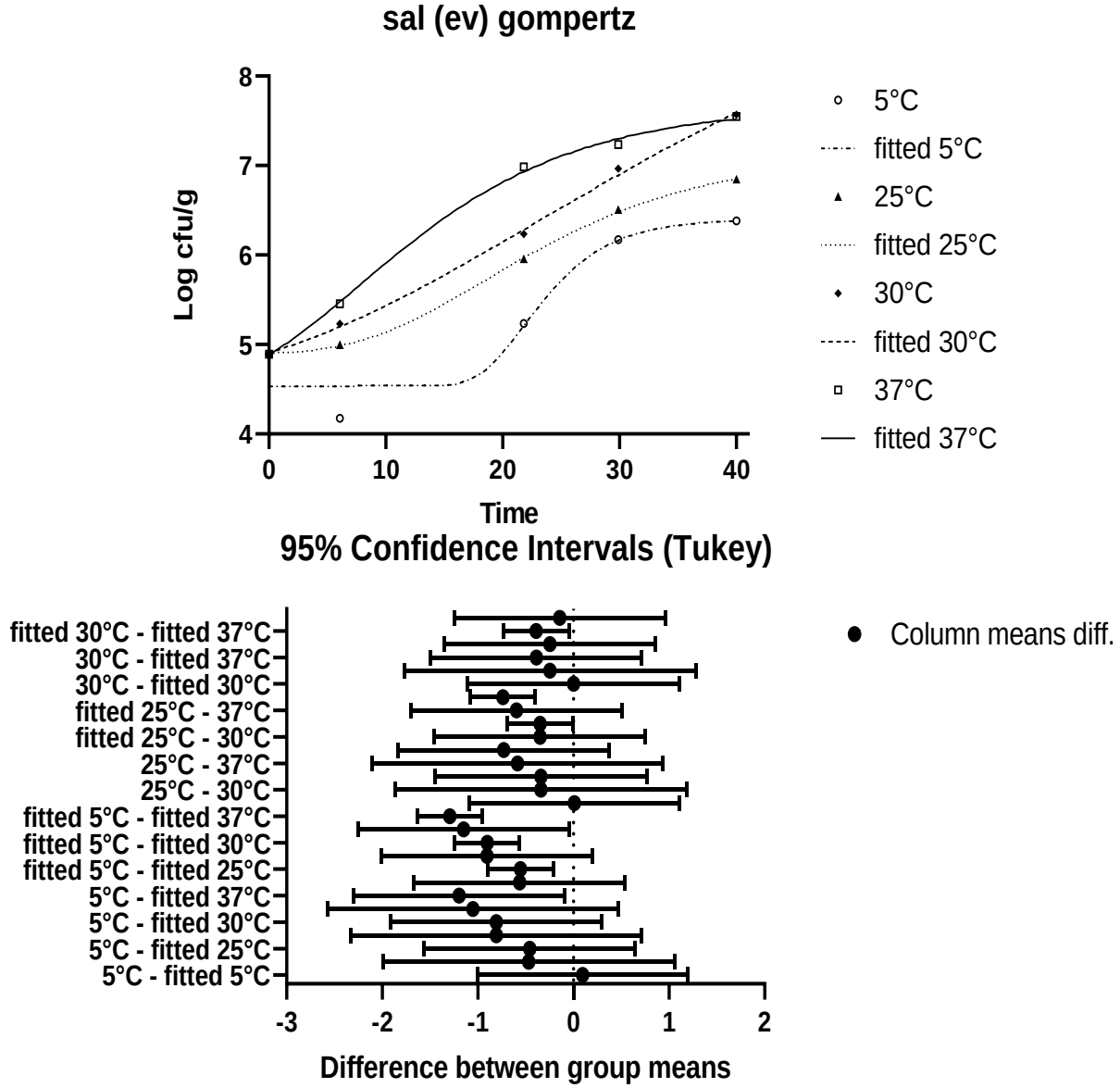


Figure 31: growth of Sal (EV5) in chicken meat at various temperatures described with Baranyi model, Logistic model,Gompertz model with their Tukey's test results.

According to the results of the statistical analysis (ANOVA) for the growth of Salmonella (EV 5) that was adjusted by the Baranyi model, at different temperatures, the p value (p= 0,6935> 0.05). for the Logistic model, the p value was (p=0,5924 >0.05). Also the Gompertz model has a p value (p= 0,5508>0.05). As for the Tukey's test results of which are shown in the (**figure 32**), where we noticed in all the models that there is no noticeable difference in growth of temperatures 5°C and 25°C, but for the temperatures 5°C and 30° we noticed a notable difference. The same thing for 5°C and 37°C but there is a little bit bigger difference compared to the latter because 30°C and 37°C have small difference between them. And for the difference between the growth at the two temperatures 25°C and 37°C shows a small significant difference.

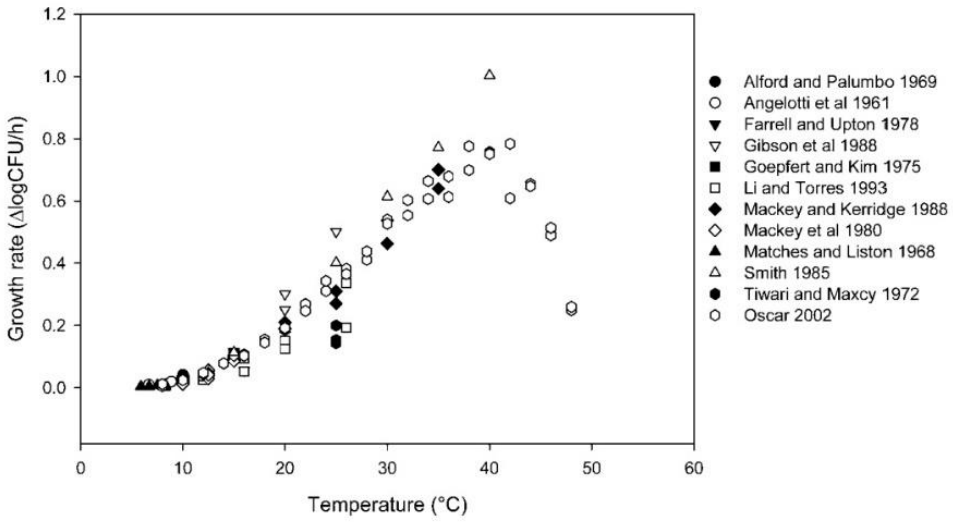


Figure 32: Salmonella growth rates at different temperatures collected from published data (SILIVIA A et al, 2008).

Table 18: Growth of Salmonella in foods at low temperatures. (Jean-Yves D'aoust, 1991)

Food	serovar	T°c	Minimum time for growth (d)	Reference
Minced chicken (b)	*S.typhimurium*	2.0	≤ 2	Baker et al.(1986)
Chicken parts (a)	*S.enteritidis*	2.0	3-6	Kim et al.(1989)
Chicken broth (b)	*S.infantis*	5.1	Not stated	Alcock(1987)
Chicken à la king (b)	Mixture (3 serovars)	6.7	4	Angelotti et al.(1961)

a: naturally contaminated.

b: artificially contaminated

Table 19: Comparison of statistics obtained from three primary models of Sal (Ev 5) the goodness-of-fit was estimated by calculating the RSS, MSE, RMSE, AIC, BIC.

T°c	Model	RSS	MSE	RMSE	AIC	BIC
5°c	Baranyi	0.254	0.254	0.504	15.627	14.065
	Logistic	0.252	0.252	0.502	15.592	14.03
	Gompertz	0.252	0.252	0.502	15.592	14.03
25°c	Baranyi	0	0	0.001	-53.491	-55.054
	Logistic	0.006	0.006	0.078	-3.056	-4.618
	Gompertz	0.002	0.002	0.041	-9.528	-11.09
30°c	Baranyi	0.009	0.009	0.096	-0.968	-2.531
	Logistic	0.014	0.014	0.117	1.016	-0.547
	Gompertz	0.009	0.009	0.092	-1.33	-2.892
37°c	Baranyi	0.018	0.018	0.131	2.328	0.766
	Logistic	0.018	0.009	0.094	0.354	-0.826
	Gompertz	0.006	0.006	0.08	-2.759	-4.322

In **(table 19)**, there is a consistency observed between the actual data and the predictions generated by the Baranyi, Logistic, and Gompertz models, indicating their effectiveness in characterizing the growth of Salmonella (Ev5) on chicken meat. The accompanying table provides a comparison of RMSE, RSS, MSE, AIC, AICc, and BIC across these models. Remarkably, the Gompertz model demonstrates the lowest values for AIC, RMSE, RSS, MSE, and BIC at 5°C, 30°C, and 37°C. Consequently, the Gompertz model stands out as the most appropriate model for depicting the growth of Salmonella (Ab 2) in this specific experimental arrangement.

Table 20: Growth kinetics values of Sal (EV 5) in chicken meat, calculated from the Baranyi and Logistic and Gompertz equations.

T°	Growth kinetics	Baranyi	Logistic	Gompertz
5°c	N_0	4.534 ± 0.359	4,535	4,535
	μ_{max}	0.129 ± 0.121	0,375	0,395
	Lag time	16.703 ± 8.617	17,515	17,89
	N_{max}	6.38 ± 0.509	6,387	6,4
25°c	N_0	4.894 ± 0.0049	4,945	4,893
	μ_{max}	0.072 ± 0.000708	0,205	0,177
	Lag time	7.199 ± 0.214	10,11	7,789
	N_{max}	6.871 ± 0.00668	6,919	7,136
30°c	N_0	4.903 ± 0.0889	4,89	4,546
	μ_{max}	0.0717 ± 0.00795	0,162	0,176
	Lag time	2.453 ± 2.834	1,516	-0,91
	N_{max}	7.2 ± 0.119	9,133	9,512
37°c	N_0	4.897 ± 0.104	4,889	4,485
	μ_{max}	0.0937 ± 0.00846	0,226	0,255
	Lag time	7.46 ± 0.111		-2,881
	N_{max}		7,514	7,667

Data collected at 5, 25, 30, and 37 °C were utilized to develop the primary growth model of *E.coli* and Salmonella in the chicken meat. Through analyzing the previous results, we have concluded that The Baranyi, and Gompertz models exhibited superior fit to the growth data of the two bacteria in comparison to the Logistic model. A comprehensive evaluation using all the parameters mentioned previously revealed that the Gompertz model was selected as the primary model for describing the growth kinetics of *E.coli* and Salmonella in poultry.

3.2 Secondary modeling

3.2.1 The effect of butcher and fridge temperatures on Salmonella and *E.coli* growth

The maximum growth rates estimated from each primary model at different isothermal conditions were then fitted using Ratkowsky secondary model. The approximate lowest temperature (5-10°C) which Salmonella and *E.coli* can grow was given as the starting value for the parameter (T_{min}).

Similarly, the approximate highest temperature at wich Salmonella and *E.coli* can grow (42°C) was given as a starting value for the parameter (T_{max}).

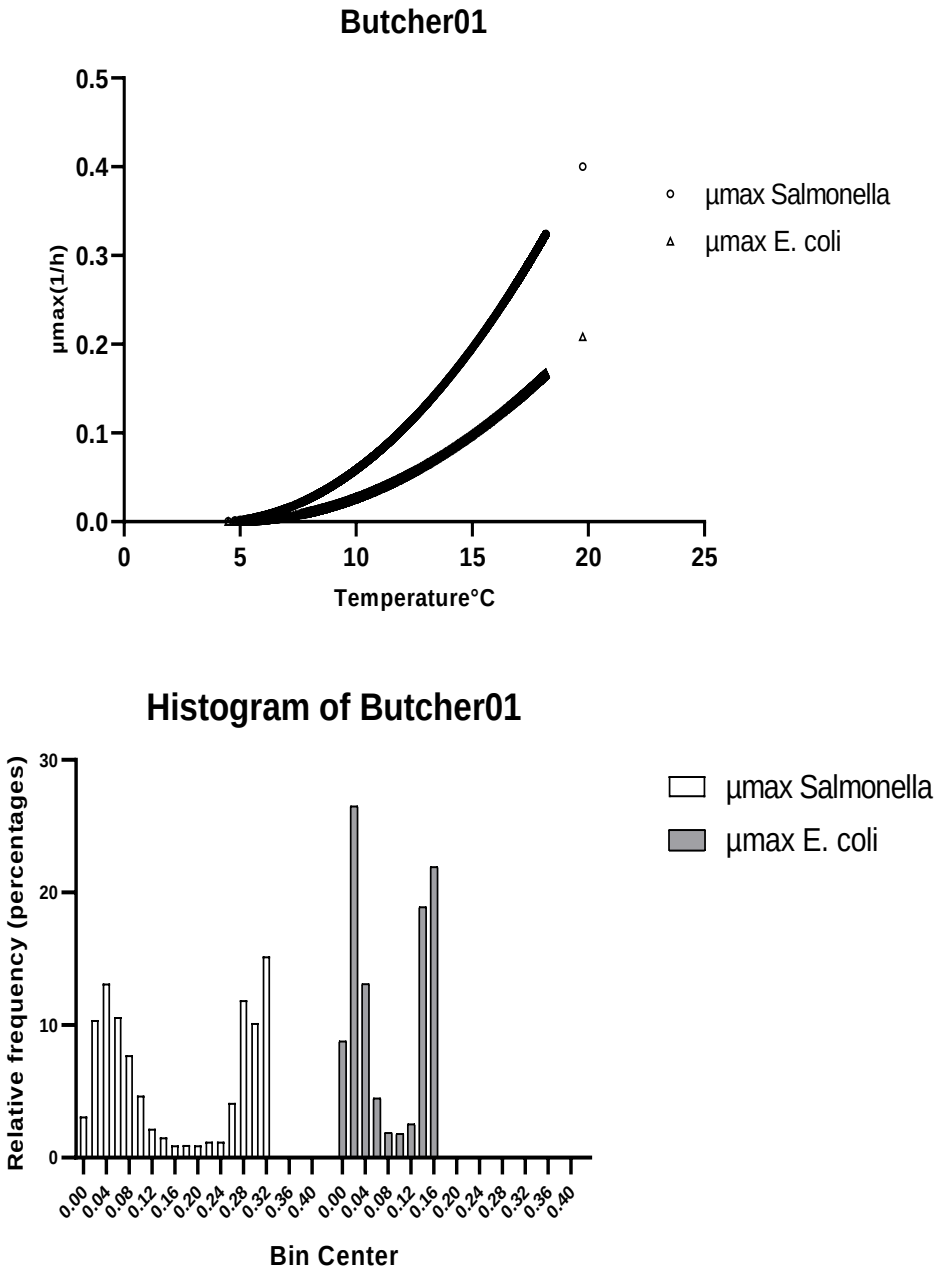
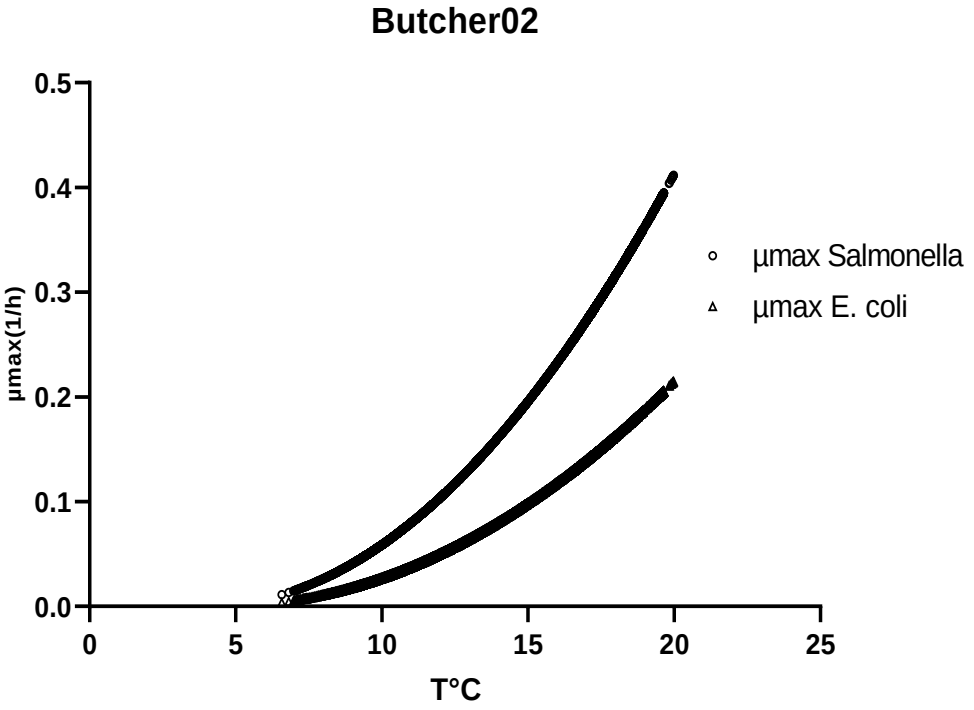


Figure 33: secondary model fit to data for maximum specific growth rate (μmax) of Salmonella and *E.coli* as a function of butcher 1 temperature.

The two graphs present data related to the growth rates of Salmonella and *E. coli* bacteria as a function of temperature, as well as a histogram of the maximum growth rates (μmax) for these bacteria (**figure 34)**.

Graph 1: Growth Rates vs. Temperature. As temperature increases, the growth rates (μmax) of both Salmonella and *E. coli* increase but Salmonella has a higher growth rate compared to *E. coli* across the temperature range. The growth rate for both bacteria is minimal at lower temperatures (around 0°C) and increases significantly as the temperature approaches 25°C, the steep increase in the growth rates, especially for Salmonella, suggests a higher sensitivity to temperature changes, making warmer temperatures more conducive to rapid bacterial growth.

Graph 2: Histogram of μmax. The histogram indicates the distribution of maximum specific growth rates for both bacteria. For Salmonella (white bars), the relative frequency peaks at higher μmax values, suggesting that higher growth rates are more common for this bacterium, for *E. coli* (grey bars), the distribution is skewed towards lower μmax values, indicating that lower growth rates are more typical. The histogram also shows the variability in the growth rates for both bacteria, with Salmonella having a wider range of higher growth rates compared to *E. coli*. Salmonella tends to grow faster and more robustly than *E. coli* as the temperature increases indicates that the environmental conditions represented (temperature range from 0 to 25°C) are more favorable for the rapid growth of Salmonella compared to *E. coli*.



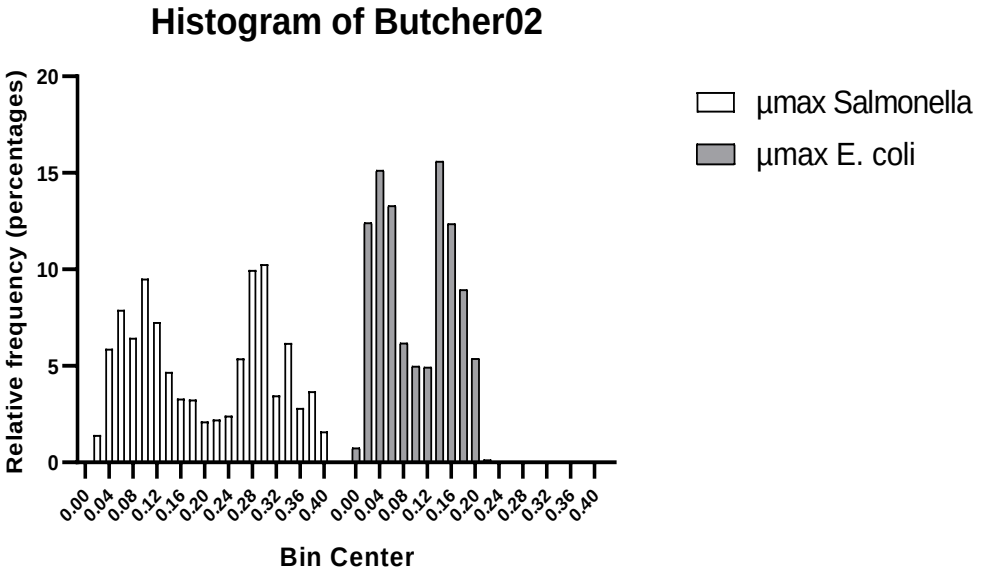


Figure 34: secondary model fit to data for maximum specific growth rate (µmax) of Salmonella and *E.coli* as a function of the second butcher temperatures.

Graph 1: Growth Rate vs. Temperature. The growth rate of *E. coli* increases with temperature. The curve is steeper at higher temperatures, suggesting a more rapid increase in growth rate as temperature rises. Similar to *E. coli*, Salmonella shows an increasing growth rate with temperature. The curve is steeper, indicating a more pronounced increase in growth rate at higher temperatures compared to *E. coli*. Both bacteria show negligible growth rates at low temperatures (below approximately 10°C), the growth rate increases significantly between 10°C and 25°C for both bacteria. However, Salmonella appears to have a higher (µmax) at each temperature point compared to *E. coli*.

Graph 2: Histogram of Relative Frequency. The (µmax) values for Salmonella are spread across a range of bins, indicating a wide variability in growth rates. The highest relative frequencies are around specific central bins, but the distribution is more even compared to *E. coli*. But the (µmax) values for *E. coli* show higher relative frequencies in fewer bins, suggesting a narrower range of optimal growth rates. Peaks are visible in specific bins, indicating optimal growth conditions are more common or significant for *E. coli.*

The peaks in *E. coli*'s distribution indicate that certain conditions favor its growth more significantly than others, as seen by the higher concentration of higher relative frequencies in specific bins, Salmonella's more evenly spread distribution indicates it can adapt to a broader range of conditions, but does not have as pronounced peaks as *E. coli*.

The histogram showing that *E. coli* has more pronounced optimal growth conditions, while Salmonella is more adaptable. This suggests that in practical applications, controlling conditions to specifically target *E. coli*'s optimal growth can be more straightforward, while a broader range of controls might be necessary for Salmonella**. (figure 35)**

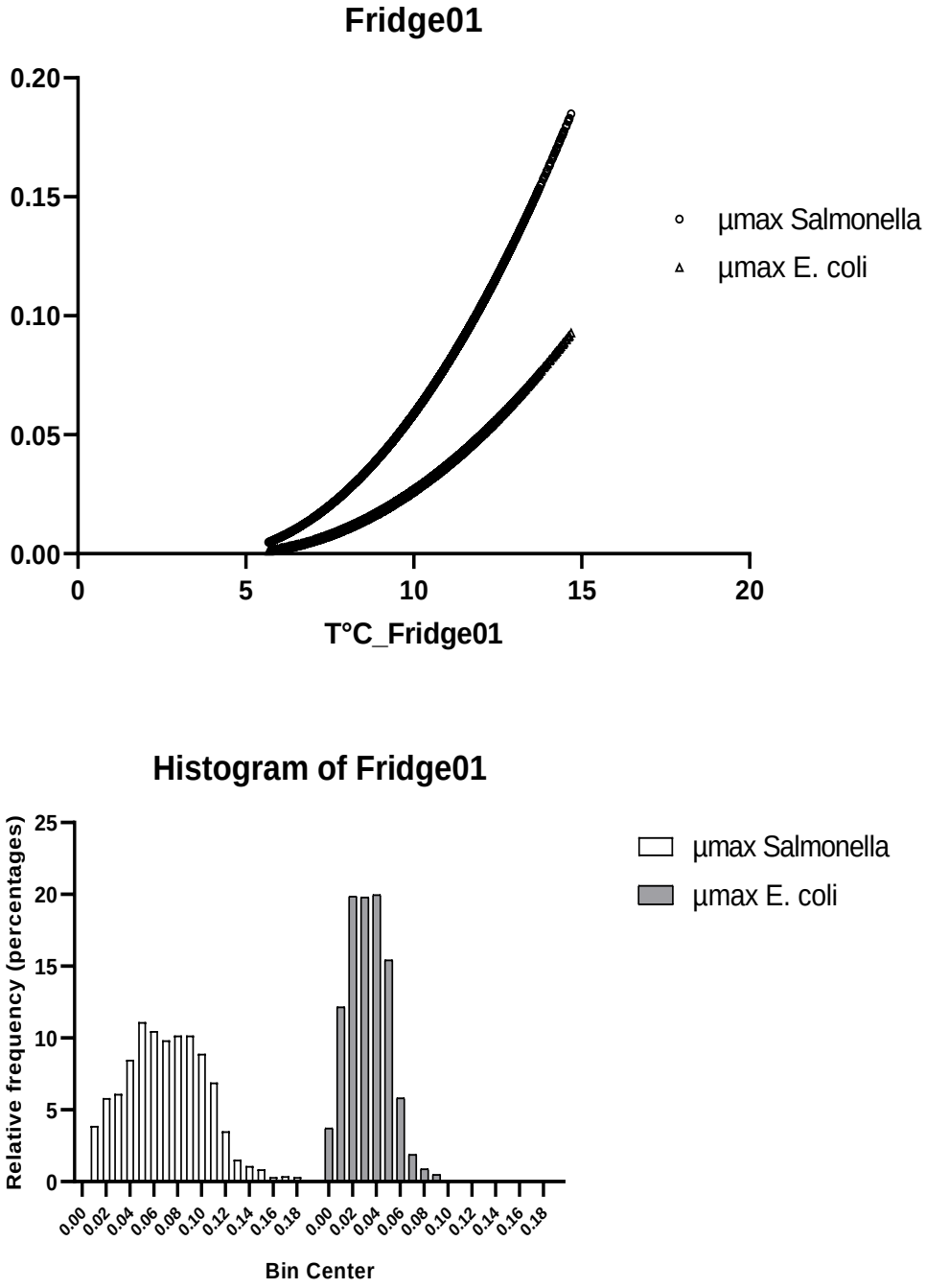
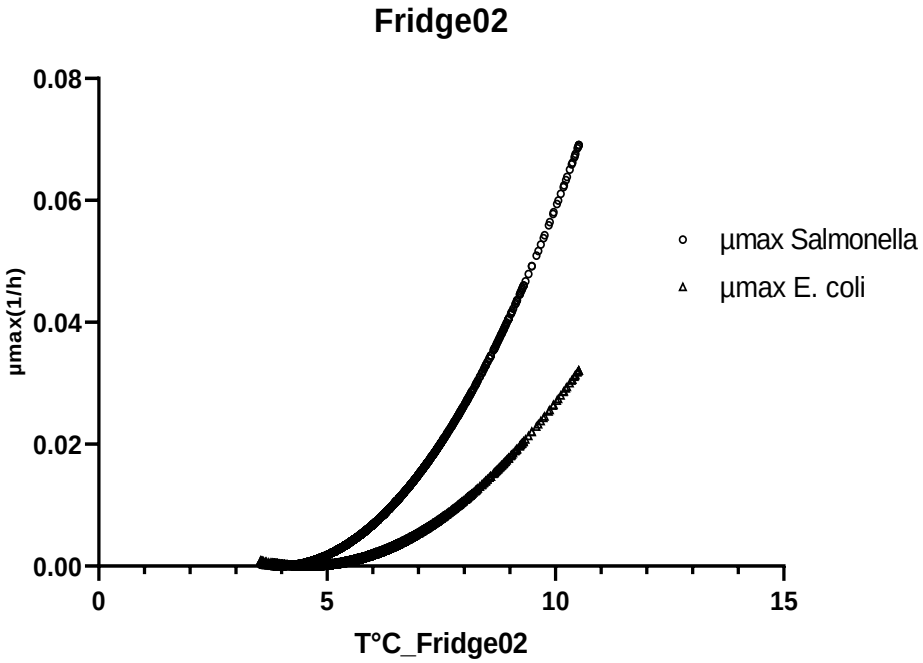


Figure 35: secondary model fit to data for maximum specific growth rate (µmax) of Salmonella and E.coli as a function of the first fridge temperature.

Graph 1: Growth Rates vs. Temperature. This graph displays the maximum growth rates (μmax) of Salmonella and *E. coli* as a function of the temperature inside the first fridge (°C).

Both Salmonella and *E. coli* growth rates increase with temperature but Salmonella (μmax) shows a higher growth rate compared to *E. coli* at the same temperatures. At lower temperatures (0-5°C), the growth rates for both bacteria are minimal, indicating that refrigeration significantly slows down bacterial growth, but as the temperature approaches the higher range (15-20°C), the growth rates for both bacteria increase sharply, which suggests that these conditions are more conducive to bacterial proliferation

Graph 2: Histogram of Growth Rate Frequencies. *E. coli* (μmax) has a more frequent occurrence of higher growth rates compared to Salmonella which her distribution is more spread out and tends to be lower than *E. coli*'s, as indicated by the lighter bars being more prevalent at lower growth rates. The peak frequency for *E. coli* growth rates is higher and occurs at higher growth rate intervals compared to Salmonella, reinforcing that *E. coli* generally grows faster under the same conditions. So, *E. coli* grows more rapidly than Salmonella at any given temperature within the tested range (**figure 36).**



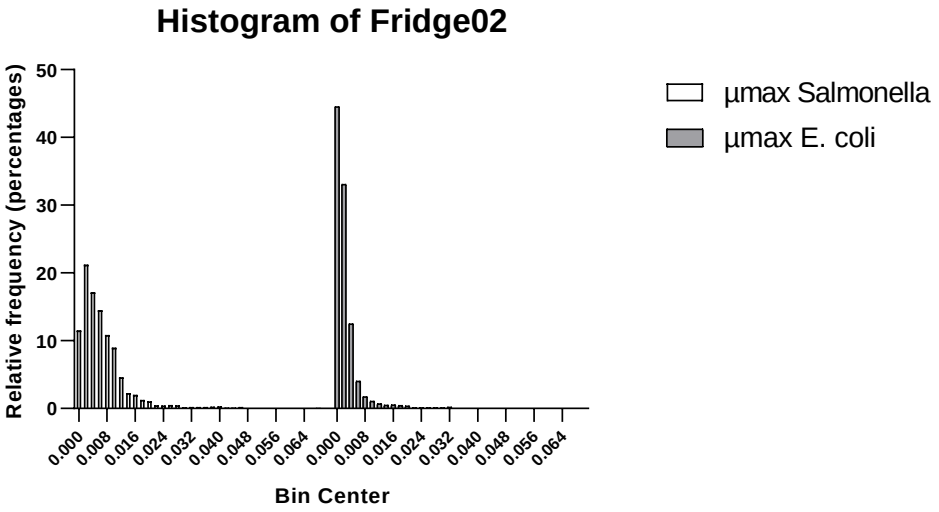


Figure 36: secondary model fit to data for maximum specific growth rate (μmax) of Salmonella and E.coli as a function of the second fridge temperature.

Graph 1: Growth Rate vs. Temperature. Both Salmonella and *E. coli* growth rates increase with temperature but Salmonella has a higher growth rate compared to *E. coli* at any given temperature, the growth rates for both bacteria are almost negligible at temperatures below 5°C and start increasing significantly around 5-10°C. Salmonella shows a steeper increase in growth rate compared to *E. coli* as temperature rises.

Graph 2: Histogram of Growth Rates. The majority of the relative frequency for (μmax) values for both bacteria is concentrated at lower growth rates. *E. coli* has a distribution that extends to higher growth rates compared to Salmonella. The highest relative frequency for Salmonella (μmax) values is clustered around 0.002-0.004 (1/h), while for *E. coli* it is slightly higher but also shows a wider distribution, indicating a greater variability in growth rates.

E. coli appears to be more sensitive to temperature changes compared to Salmonella, showing higher growth rates at the same temperatures. This could suggest that *E. coli* might pose a higher risk of proliferation in slightly warmer conditions within the fridge.

The histogram indicates that under typical conditions within Fridge 02, the growth rates for both bacteria are generally low, but *E. coli* has a potential to achieve higher growth rates more frequently than Salmonella. This could be due to differences in their metabolic capacities and responses to environmental conditions **(figure 37).**

3.2.1.1 Differences Between Butcher and Fridge Datasets:

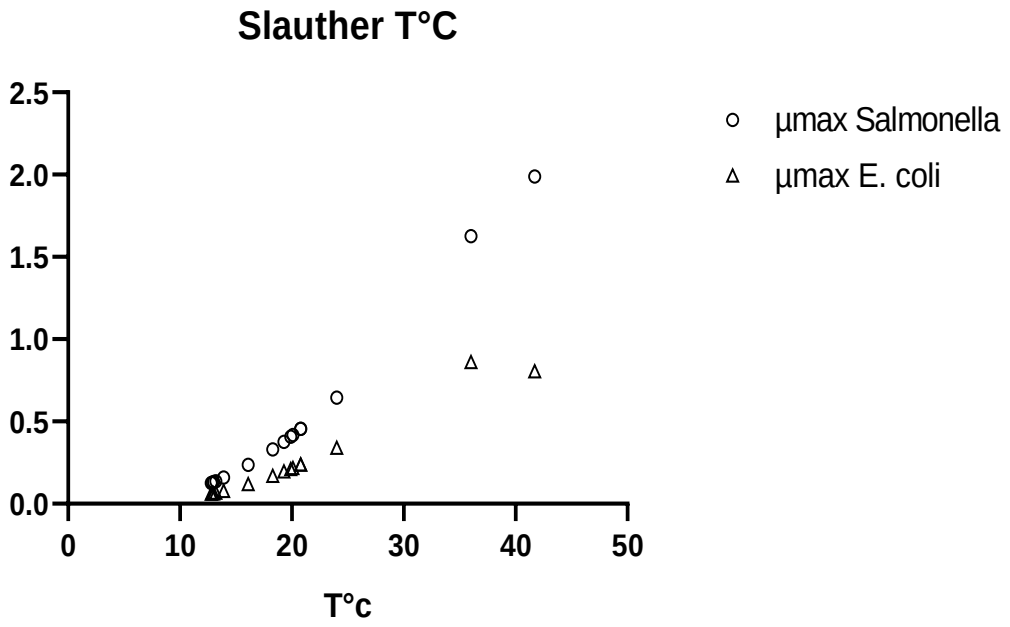
Curves: Butcher Datasets: Both Butcher 01 and Butcher 02 show similar exponential growth patterns, with Butcher 02 displaying higher growth rates overall compared to Butcher 01.

Fridge Datasets: Both Fridge 01 and Fridge 02 also display exponential growth, but the rates are lower compared to the Butcher datasets. Fridge 02 shows the lowest growth rates among all datasets.

Histograms: Butcher Datasets: in butcher 01 (µmax) for *E. coli* shows higher frequency in higher bins compared to Salmonella. Butcher 02 is similar pattern as Butcher01, but with more spread, indicating higher variability.

Fridge Datasets: in fridge 01 (µmax) for *E. coli* has distinct peaks and a broader distribution compared to Salmonella. And for fridge 02 Both (µmax) for Salmonella and *E. coli* have narrow distributions, with a pronounced peak in lower bins, indicating lower and less variable growth rates.

In summary, these graphs provide valuable insights into how temperature affects the growth rates of Salmonella and *E. coli*, with clear implications for food safety and microbiological control in environments where these pathogens may be present. The comparison suggests that temperature significantly affects the growth rates of Salmonella and *E. coli*, with *E. coli* consistently having higher (µmax) across all conditions. The Fridge conditions (lower temperatures) result in reduced and less variable growth rates compared to Butcherconditions (higher temperatures).



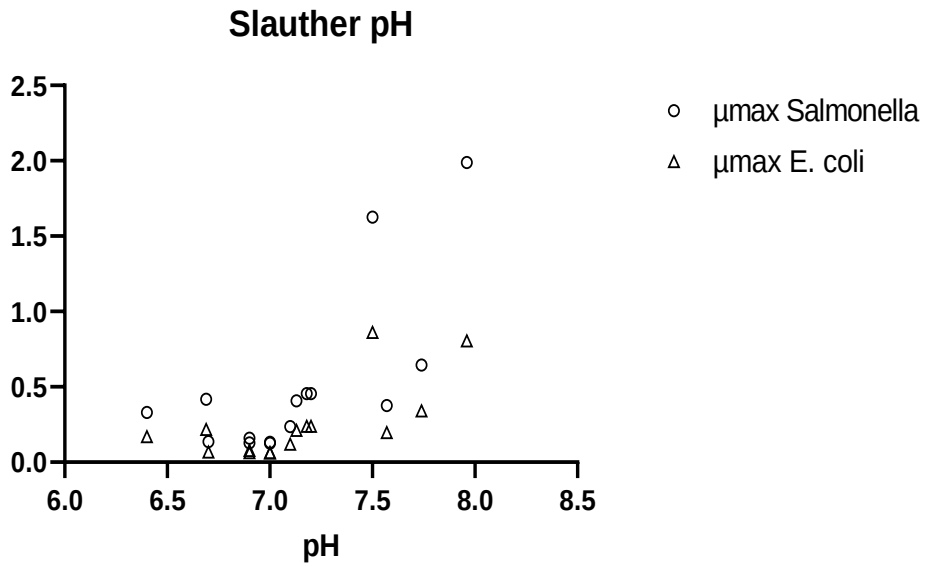


Figure 37: The two graphs display the maximum growth rates (µmax) of Salmonella and E. coli in relation to temperature (°C) and pH.

At lower temperatures (0-20°C), the growth rate of Salmonella is relatively low, typically below 0.5. As temperature increases to around 35-40°C, the growth rate of Salmonella significantly increases, reaching values close to 2.0. There appears to be an optimal temperature range around 35-40°C for the maximum growth rate of Salmonella (**figure 38**).

The growth rate of *E. coli* also shows a similar trend to Salmonella, with low growth rates at lower temperatures. The growth rate increases as the temperature rises, but the maximum growth rate for *E. coli* seems to peak at slightly lower values compared to Salmonella. Optimal growth temperatures for *E. coli* seem to be in the range of 30-35°C.

The work by Doyle and Schoeni (1984) and Palumbo et al. (1977) highlights the effects of temperature on the growth of *E. coli* and Salmonella, showing varying responses at different temperatures.

Salmonella has a wide range of growth rates across the pH spectrum, there is a noticeable peak in growth rate around pH 7.0-7.5, indicating that neutral to slightly alkaline conditions are optimal for Salmonella growth. The growth rate is lower at more acidic (pH 6.0-6.5) and more alkaline (pH 8.0-8.5) conditions (**figure 38**).

E. coli shows a similar pattern to Salmonella, with a higher growth rate around neutral pH (7.0-7.5). The growth rate decreases both in more acidic and more alkaline conditions, but the decrease is more pronounced at the alkaline end (above pH 7.5).

These trends are consistent with known growth characteristics of these bacteria, where temperature and pH are critical factors influencing their proliferation.

Studies by Foster et al. (1991) and Lin et al. (1996) investigate the impact of pH on bacterial growth, showing that pH significantly affects both *E. coli* and Salmonella but in different ways. Our data, which shows significant differences when comparing pH impacts on N(t) Salmonella and N(t) *E. coli*, is consistent with these findings, highlighting that pH is a crucial factor in microbial growth dynamics. But since its effect is not significant, it is not taken into consideration.

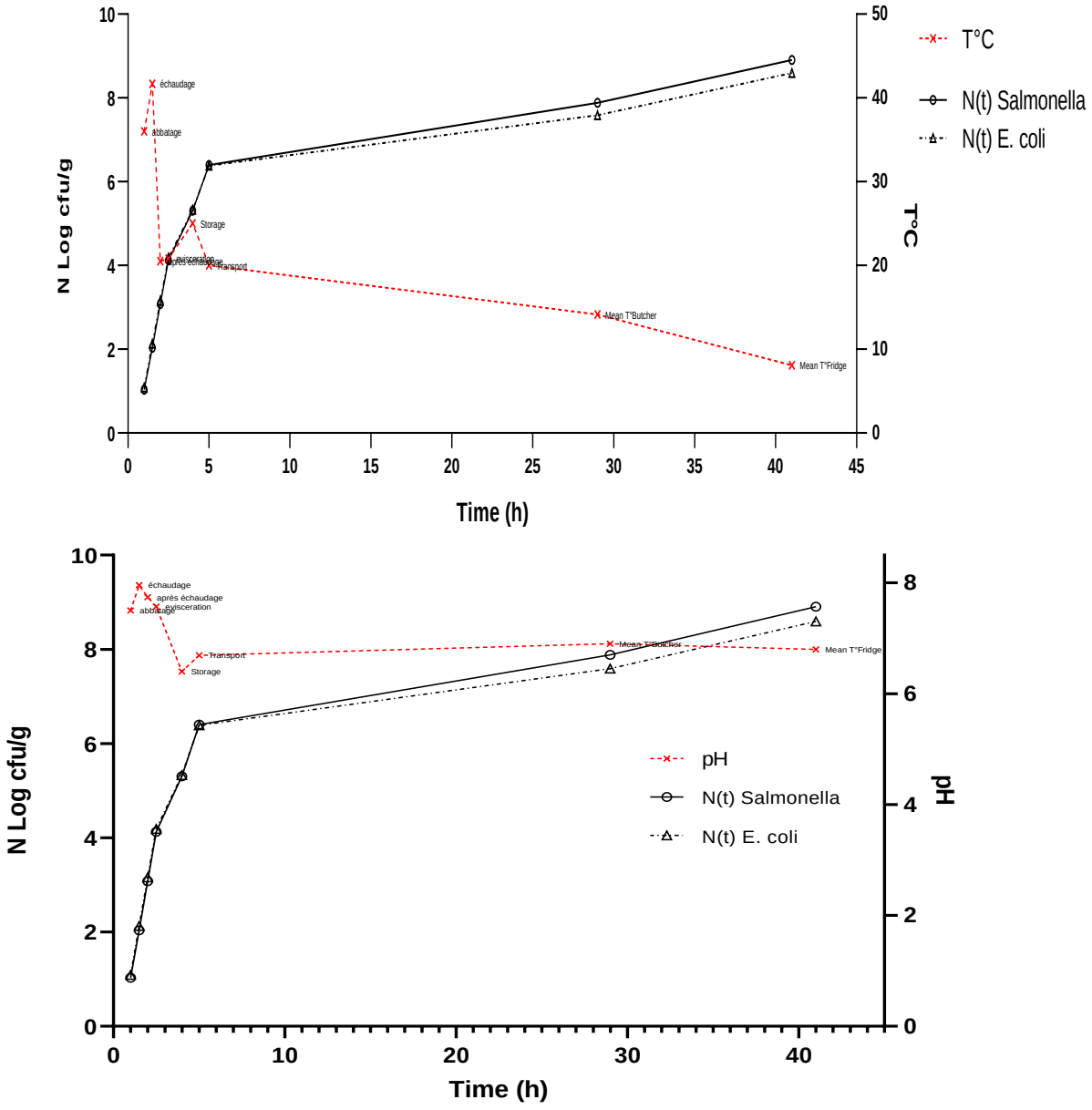


Figure 38: the growth dynamics of Salmonella and E. coli under varying conditions over a period of 40 hours. Each graph includes different parameters that potentially influence bacterial growth: temperature (left graph) and pH (right graph).

Graph 1: Influence of Temperature

Temperature starts around 42°C and drops to around 28°C by 40 hours. This gradual decrease in temperature could impact bacterial growth rates, as both Salmonella and *E. coli* have optimal growth temperatures **(figure 39).**

By assuming an initial population of Salmonella is around 1 log cfu/g, increase rapidly to around 6.5 log cfu/g within the first 5 hours, growth continues at a slower rate, reaching around 8.5 log cfu/g by 40 hours. And by assuming the initial population of *E.coli* also around 1 log cfu/g, increase rapidly to around 6.5 log cfu/g within the first 5 hours, growth continues slowly, reaching just above 8 log cfu/g by 41 hours.

Both bacteria show rapid initial growth likely due to the favorable temperature at the start, which is close to their optimal growth temperatures. As the temperature decreases, growth rates slow down, indicating the temperature drop negatively impacts the growth of both bacteria.

Graph 2: Influence of pH

pH starts around 7.4 and drops slightly to around 6.7 within the first 10 hours, it remains relatively constant around 6.7 for the remainder of the experiment (**figure 39**).

Initial population of Salmonella is around 1 log cfu/g, increasing rapidly to around 6.5 log cfu/g within the first 5 hours. Growth continues at a slower rate, reaching around 8.5 log cfu/g by 40 hours.

Initial population of *E.coli* is also around 1 log cfu/g, increasing rapidly to around 6.5 log cfu/g within the first 5 hours. Growth continues slowly, reaching just above 8.5 log cfu/g by 40 hours.

Both bacteria exhibit rapid growth initially, likely due to the near-neutral pH (~7.4), which is ideal for their growth. The slight drop in pH does not seem to significantly hinder their growth, as both bacteria continue to grow, albeit at a slower rate after the initial phase.

Both graphs show a rapid increase in bacterial populations within the first 5 hours, indicating that the initial conditions (temperature around 42°C and pH around 7.4) are highly favorable for bacterial growth. The growth rate decreases after the initial phase in both scenarios, suggesting that suboptimal conditions (lower temperature and slightly acidic pH) negatively affect the growth rates of both Salmonella and *E. coli*. Salmonella seems to reach a higher population density compared to *E. coli* under the same conditions, indicating it might be more robust or have a slightly higher growth rate under the tested conditions.

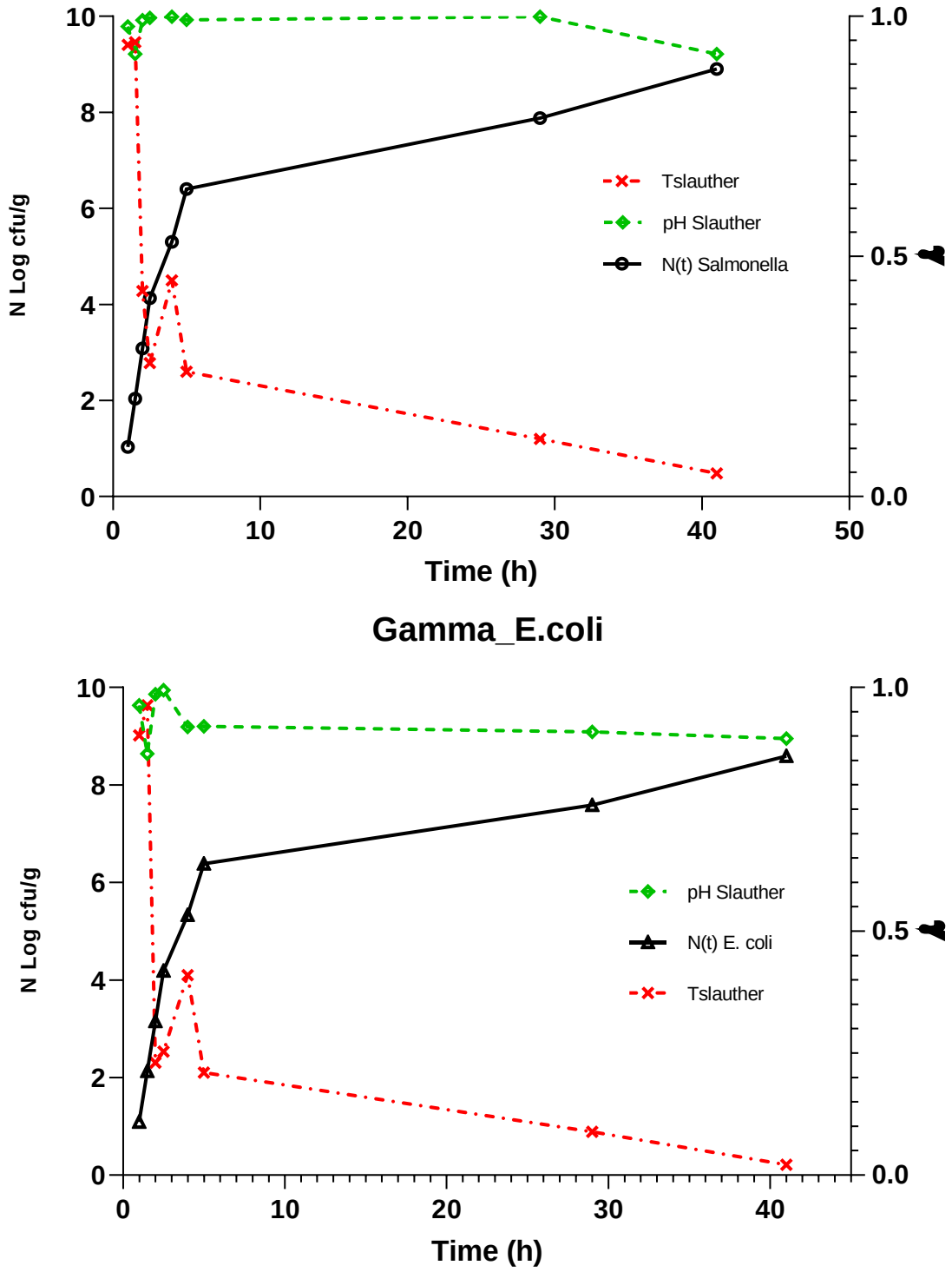
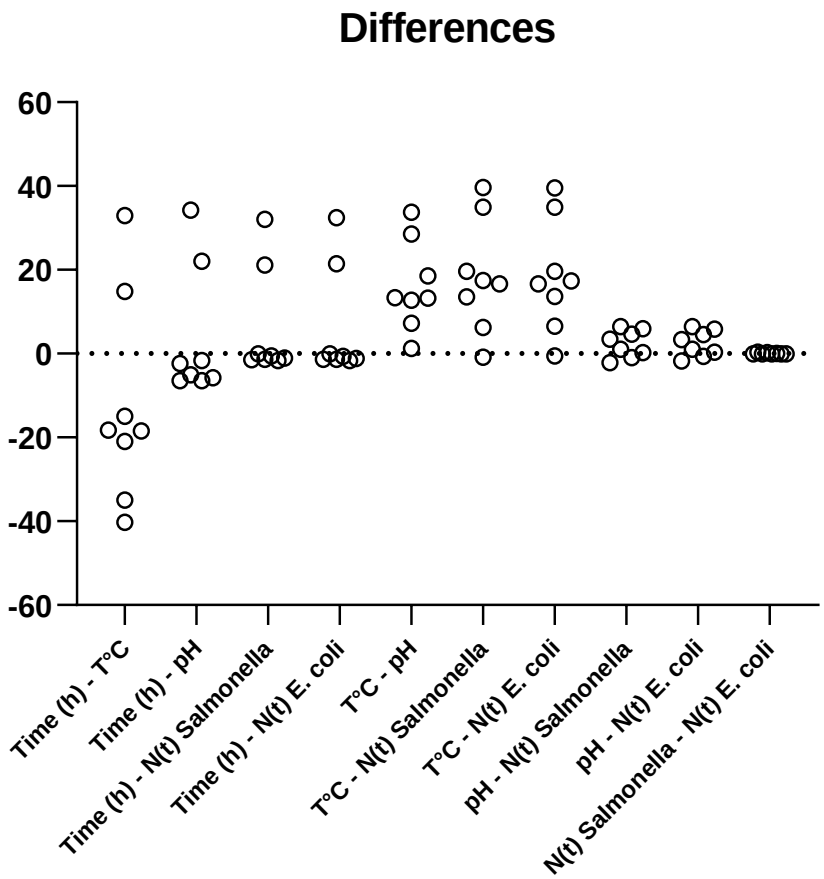


Figure 39: the dynamics of bacterial populations under different conditions over a period of 40 hours.

The black line shows the population density (N, in Log CFU/g) of Salmonella over time. Initially, there is a sharp increase in population, indicating rapid bacterial growth. After reaching a peak, the population stabilizes or slightly decreases, suggesting a plateau phase where growth rate and death rate are balanced.

The red line represents γT (the effect of temperature on the increasing of N (Log CFU/g)) and the green line the γpH (the effect of pH on the increasing of N(Log CFU/g)), this study found that the populations of Salmonella and *E. coli* (Nmax) could reach 8.9 log CFU/g and 8.5 log CFU/g, respectively, after 41 hours, assuming an initial population (N0) of 1 log CFU/g. Results showed that T°C had a significant effect on the two bacterial growth because when γT was close to one (Topt) ($\gamma T \approx 0.96$) there was no effect on bacterial growth (the increase was rapid as shown in the (**figure 40**) but when the γT approaching to zero the effect of temperature appeared when the speed of bacterial growth decreases. In contrast, pH had a minimal effect on bacterial growth because γpH was ≈ 1 in all the slaughtering process.



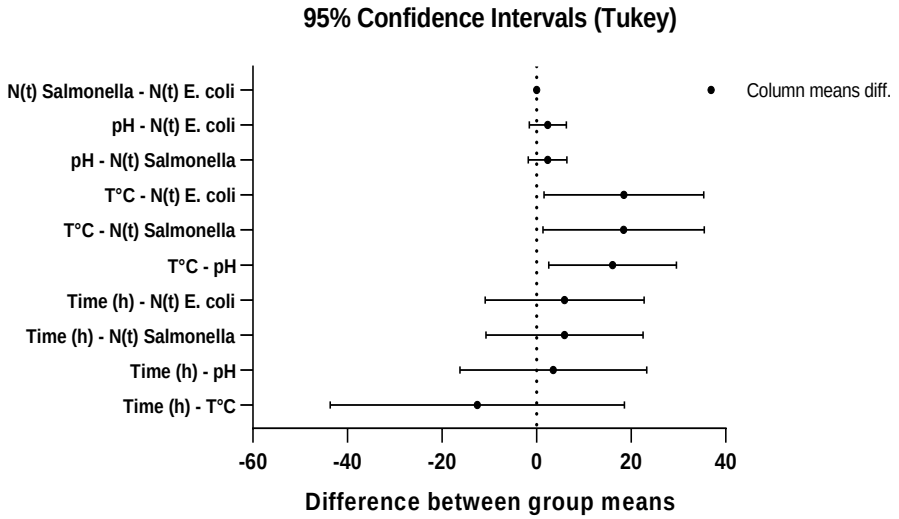


Figure 40: Tukey's test results

The graphs provided represent the results of a Tukey's test, a post-hoc analysis used to identify significant differences between different conditions of growth after an ANOVA has determined.

About the (Tukey) graph, if a confidence interval does not cross zero, the difference between the means of the two groups is statistically significant at the 95% confidence level. If a confidence interval crosses zero, the difference is not statistically significant **(figure 41).**

When N(t) Salmonella – N(t) E. coli crosses zero, it signifies a statistically insignificant difference in means between these two groups, suggesting no effect on each other. Conversely, the effects of pH, temperature (T°C), and time on N(t) of Salmonella and *E. coli* vary: pH near zero indicates minimal impact on bacterial growth, while non-zero T°C significantly affects growth. Time also affects growth but less than T°C. The interaction of Time (h) and pH shows a slight significant difference in means, indicating some influence. Conversely, Time (h) and T°C interaction exhibits a large significant difference, indicating a substantial influence. Studies like Smith et al. (2003) and Zhao et al. (1993) analyze bacterial growth over time, found that time affects bacterial growth, our data showing non-significant differences over time aligns with these studies, indicating that time alone, without varying other factors like pH or temperature, may not lead to significant differences in growth rates. Lastly, T°C and pH interaction shows a significant difference, suggesting a notable mutual influence. pH does not directly affect temperature, but it can influence biochemical processes that are temperature-sensitive (White, D. 2007).

Studies like those by Flint et al. (2005) and Lammerding et al. (1999) have analyzed the growth rates of Salmonella and *E. coli* under different conditions, finding significant differences in growth

patterns depending on environmental factors like pH and temperature. These studies often find that *E. coli* can grow more rapidly in a wider range of conditions compared to Salmonella, which aligns with our observation of significant differences between N(t) Salmonella and N(t) *E. coli*.

Conclusion

Detection of *Escherichia coli* and Salmonella in food poses significant health risks and serves as a crucial indicator of cross-contamination. Salmonella, commonly found in chicken meat, was prevalent in all butcher shop samples and in 47% of slaughterhouse samples according to our study.

We utilized data collected at temperatures of 5, 25, 30, and 37 °C to develop primary growth models for *E.coli* and Salmonella in chicken meat. Our analysis concluded that Gompertz models provided the best fit to the growth data for both bacteria, based on comparisons involving RMSE, RSS, MSE, AIC, AICc, and BIC. Subsequently, we used the Ratkowsky secondary model to estimate maximum growth rates at different temperatures.

The results of the dynamic analysis indicated that the optimal specific growth rates (μopt) for Salmonella and *E. coli* were 1.63±0.08 1/h and 1.05±0.06 1/h, respectively then employed the gamma concept to predict how temperature and pH affect microbial growth. Our dynamic analysis revealed that temperature significantly influences bacterial growth, whereas pH exerts a minimal effect.

The particular strength of quantitative modeling tools lies in their potential to determine the relative effect of interventions and to show the best decisions. Understanding these dynamics can inform preventive measures such as respecting the storing chicken temperature at 4°C and ensuring thorough cooking to eliminate bacteria, as well as maintaining cleanliness during food handling to prevent cross-contamination. these tools should not be considered absolute and decisions should not be based solely on modeling and can fulfil an important model prediction to the hazards and risks management.

Looking ahead, several perspectives could extend and enhance this research:

- Validate these findings across additional temperature ranges.
- Investigate the fate of these bacteria during cooking stages in home settings.
- Confirm that the FSO (food safety objectives) are adhered to the ICMSF equation.
- Evaluate the public health risk associated with consuming chicken meat.
- Integrate strategies for Salmonella and *E. coli* inactivation throughout the production process to conduct accurate exposure assessments.

Bibliography

Ahmed, A. M., Younis, E. E., Ishida, Y., Shimamoto, T. (2007). Genetic analysis of multiple antimicrobial resistance in Salmonella isolated from diseased broiler chickens in Egypt. Microbiology and immunology, 56 (8), 254–261.

Azam, Ahmadi, Parisa, Ahmadi,.Ali, Ehsani (2020). Development of an active packaging system containing zinc oxide nanoparticles for the extension of chicken fillet shelf life. Food Sci Nutr, 8 (10), 5461-5473.

Coburn, B., Guntram, A. Grassl, B. B. (2007). Salmonella, the host and disease: a brief review. Immunol Cell Biol, 85(2),112.

Calnek, B.W., Barnes, H.J., Beard, C.W., McDougald, L.R., Saif, Y.M. (1997). Diseases of poultry. Avian Pathology, 2008, 37 (04), 355-366.

Barnes (1997). Characterization of Two Escherichia coli Isolates Associated with Poult Enteritis and Mortality Syndrome.

Barrow, G. I, and Feltham,.R, K,. A. (2003). Cowan and Steel's Manual for the identification of the Medical Bacteria. American Journal of Microbiological Research, 5 (6), 138-141.

Barrow, G. I. Feltham,R.K. A. (2003). Cowan and Steel's Manual for the identification of the Medical Bacteria. American Journal of Microbiological Research, 5 (6) ,138-141.

Benyoucef., Bouzegag. (2006). Resultats D'etude De La Qualite De La Viande De Deux Races Cameline. In the Algerian Annals of Agronomy, 27 (1), 37-53.

Bey, F.D., Aniel, Pérez, M. (2009). *Escherichia coli* genes affecting recipient ability in plasmid. BMC Genomic, 10 (47) ,118-147.

Biesalski, H.K. (2005) . Meat as a Component of a Healthy Diet—Are There Any Risks or Benefits if Meat Is Avoided in the Diet? Meat Science, 70 (21), 509-524.

Biswas, P. K., Uddin, G. M. N., Barua, H., Roy, K., Biswas, D., Ahmed, M. (2004). Prevalence and characteristics of Salmonella in commercial poultry in Bangladesh. Preventive Veterinary Medicine, 83 (9), 260–271.

Bovill, R., Bew, J., Baranyi, J., and Cox, L. (2001). Measurements and predictions of growth for Listeria monocytogenes and Salmonella during fluctuating temperature II. Rapidly changing temperatures. International of Food Microbiology ,67 (23), 131-137.

Carl, M., Schroeder's. (2004). A DNA microarray for identification of virulence and antimicrobial resistance genes in Salmonella serovars and Escherichia coli. Mol Cell Probes, 19 (3),195-201

Christian, L, Lauber. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology, 75 (56), 5111-5120.

Danilo, M.A,. Lo, Fo,. Wong, Mary,. E, Patrick,. Norma Binsztein, Anna , Cieslik,. Thongchai,C., Awa, Aidara-Kane,. Andrea, Ellis, F. J,. Angulo, H. C,. Wegener. (2000). Emerg Infect Dis, 12(3),381-8.

Dar. T. (2017). Salmonella typhimurium in poultry. World's Poultry Science Journal,73 (2) ,345–354

Dhandy, K., Wardhana, A. E. P. Haskito,. Muhammad Thohawi, M., Suwaibatul A. (2021). Detection of microbial contamination in chicken meat from local markets, 14 (12), 3138–3143.

Doyle, M. P., ROMAN, D. J. (1981). Growth and Survival of Campylobacter fetus subsp. jejuni as a Function of Temperature and pH. Journal of Food Protection, 44(8), 596-601.

E, Baéza. (2022). Production factors affecting poultry carcass and meat quality. the journal Animal, 16 (15), 100-331.

Edens, F.W., Qureshi, R.A., Parkhurst, C.R., Qureshi, M.A., Havenstein, G.B., Casas, I.A. (1997). Characterization of two Escherichia coli isolates associated with poult enteritis and mortality syndrome. Poult Science, 76 (12),1665-73.

El Groud, R., Zerdoumi, F., Benazzouz, M., Grif, K., Berger, J., Hess, C., Hess, M. (2008). Prevalence and antibiotic resistance of Salmonella isolated from broiler carcasses in Algeria. Vet World, 13(10), 2070–2084.

Emody, L., Kerényi, M., Nagy, G. (2003). Virulence factors of uropathogenic Escherichia coli. Int J Antimicrob Agents, 2,29-33.

F, W., EDENS, R., A, QURESHI., C, R., PARKHURST, M., A, QURESHI., G,B., HAVENSTEIN, and I, A., CASAS .(1997).Characterization of Two Escherichia coli Isolates Associated with Poult. Poult Sci,76 (12), 1665-73.

Food and Agriculture Organization (FAO). (1997). Foodborne Pathogens: Microbiology of Food.

Geneva. (2015). Codex Alimentarius Commission.

H,V,. S,Chauhan,. And S,Roy.(1996).Poultry Diseases, Diagnosis and Treatment. New Age International, 45 (8), 432– 444.

Habib-Ur-Rehman, F., Khan, S. A., Ashfaq, M. (2004). Incidence of Salmonella in poultry and poultry environment in Karachi, 86 (5), 167-178.

Heshu, S. R., Bakhtyar, M. M., H. O. (2018). Review of History, Definition, Classification, Source, Transmission, and Pathogenesis of Salmonella. Journal of Zankoy Sulaiman,20(4), 11-20.

Holmberg, S. D., Wells, J.G., Cohen, M.L. (1984). Animal-to-man transmission of antimicrobial-resistant Salmonella. Science, 225(4664), 833-5.

Hyeon, J.Y., Chon, J.W., Hwang, I.G., Kwak, H.S., Kim, M.S., Kim, S.K., Choi, I.S., Song, C.S., Park, C., Seo, K.H. (2011). Prevalence, antibiotic resistance, and molecular characterization of Salmonella serovars in retail meat products. Food Prot.74(1),161-6.

I, Mbata. (2004). Poultry meat pathogens and its Control. Environ Microbiol, 75 (15), 5111.

J D, Greig. (2009). Analysis of foodborne outbreak data reported internationally for source attribution. International Journal of Food Microbiology,130, 77–87.

J, E., Leonard, M., H, Saier,.Jr. (1981). Genetic dissection of catalytic activities of the Salmonella typhimurium mannitol enzyme II. J Bacteriol, 145(2), 1106-9.

Jackson, R.B. (2006) . The diversity and biogeography of soil bacterial communities. Department of Biology & Nicholas School of the Environment and Earth Sciences, 103(3), 626–631.

John, Bassett., Maarten, Nauta.,Roland, Lindqvist., and Marcel, Zwietering.(2012).TOOLS FOR MICROBIOLOGICAL RISK ASSESSMENT.

Juneja, V.K. (2009) · Mathematical Modeling of Growth of Salmonella in Raw Ground Beef. International Journal of Food Microbiology, 131 (54), 106-111.

Karen, C,. Carroll (2019). Manual of Clinical Microbiology.

Kis. Robertson., et al. (2012). Foodborne Outbreaks Reported to the U.S. Food Safety and Inspection Service. Fiscal Years 2007 through 2012.Journal of Food Protection, 79(3),442-447.

Kolmos, A. (2009). Triple Sugar Iron (TSI) agar for differentiating Enterobacteriaceae. American society of microbiology.

L, Rosso., J, R., Lobry, S., Bajard, J.P., Flandrois. (1995). Convenient Model To Describe the Combined Effects of Temperature and pH on Microbial Growth. Appl Environ Microbiol. , 61(2), 610–616.

L, W., Riley., et al. (1983). Hemorrhagic colitis associated with a rare Escherichia coli serotype. Journal of medicine 308 (12),685-695.

M, Larbier., B, Leclercq. (1994). Nutrition and Feeding of Poultry. Institut National de la Recherche Agronomique, 76 (3),305.

Merchant, I. A., Packer, R. A. (1967). Veterinary Bacteriology and Virology. Ames Iowa.

Michael, S., Donnenberg1., Thomas, S., Whittam. (2001). Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic Escherichia coli. Journal Clinic Investi,107 (5), 539–548.

Mohammed, A. (2011). Theoretical Development and Applications of Rhotrices. Ph.D. Thesis, Ahmadu Bello University, Zaria.

Nader, W. F., Al-Bahadily, A. M., Jameel, M. A. (2015). Prevalence and antimicrobial susceptibility of Salmonella species in cattle in Baghdad city, Iraq. The Iraqi journal of veterinary medicine, 37 (1), 96-101.

Natalia , Pshenichnaya.,a,Irina., Lizinfeld,a Karina ., Umbetova,b., Yulia, Konnova.,c, Galina Gopatsa.,a Ainash , Kuandykova.,d, and Balnur, Omarovad.(2023). Salmonella Reading: A rare case of generalized salmonellosis in non-endemic region, 33 (13), 65-78.

Neelja, S., Nambram, S., Singh, J., Singh, Virdi. (2019). Virulence-associated traits and in vitro biofilm-forming ability of Escherichia coli isolated from a major river traversing Northern India. Environmental Science and Pollution Research,26, 21304–21311.

Norris, D,. W, Ribbons. (1970).Methods in Microbiology.

Novais, A., Valverde, A., Machado, E, Peixe, L., Baquero, F., Coque, T.M. Prevalence of Arcobacter, Escherichia coli, Staphylococcus aureus and Salmonella species in Retail Raw Chicken, Pork, Beef and Goat meat in Osogbo, Nigeria. (2008). Clin Microbiol Infect,14 (57) ,144-53.

P, S,. KHANDEKAR, I. D. P,. BURMA, S. K, TANEJA,. M, CHAKRAVORTY (1975). Transport in Bacteriophage P22-Infected Salmonella typhimurium. J Virol, 16 (1),101-6.

Panisello, P.J., Rooney, R., Quantick, P.C., Stanwell-Smith, R. Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. Int J Food Microbiol, 59 (3), 221-34.

R ,Cantón (2008). Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Sience,5(1), 19-20.

Rabie, N. S., Sayed, M. M., Abd El-Aziz, N. K., Hamed, S. R. (2012). Prevalence of Salmonella serovars in chicken and human in Sharkia Governorate, Egypt. Journal Pre-proof, 100 (1), 220-234.

Richard, K., Gast,. Robert, E,. Porter, Jr.(2020). Salmonella Infections Diseases of Poultry. chief David E.

Sagar, A. (2022). Biochemical Test and Identification of *Salmonella Typhi*. Food Control 17 (4,1), 207–212.

Shafini., Sahan, G., David, M., Walid., Q. Alali. (2017). Salmonella prevalence associated with chicken parts with and without skin from retail establishments in Atlanta metropolitan area, Georgia. Food Control ,73 (21) ,462-467.

Soomro, A. H., M. A. Arain, M. Khaskheli, B. Bhutto, H. (2002).Isolation of Escherichia Coli from Raw Milk and Milk Products in Relation to Public Health Sold under Market Conditions at Tandojam. Journal of nutrition, 1(3), 151-152.

Sukhuntha, O,. Pravate, T,. Woranut, K,. Kooranaee, T,. Larry, E. E. (2012). Model of inactivation of *Campylobacter jejuni* in poultry scalding. Journal of Food Engineering. International Journal of Food Microbiology, 110 (1), 38-43.

Tiabaijuka, B., Gashe, B. A., Mpuchane, S. F. (2003). Prevalence of Salmonella in meat samples from butcheries in Gaborone, Botswana. Foodcont, 17 (3), 207-212.

Zschock, M., Hamann, H. P., Kloppert, B., Wolter, W. (2000). Shiga-toxin producing *Escherichia coli* in feces of healthy dairy cows, sheep and goats: prevalence and virulence properties. Microbiology, 31 (3,1), 203-208.

Complementary Data

Table: composition of culture mediums

mediums	composition	Quantity
TSI	Peptone Beef extract Yeast extract Lactose Sucrose Glucose Ferrous sulfate Sodium thiosulfate Phenol red Agar Distilled water	20 g 3 g 3 g 10 g 10 g 1 g 0.2 g 0.3 g 0.024 g 12-15 g 1 liter
SS	Peptones (beef and casein) Lactose Bile salts (oxgall) Sodium citrate Sodium thiosulfate Ferric citrate Brilliant green Neutral red Agar Distilled water	5 g 10 g 8.5 g 10 g 8.5 g 1 g 0.00033 g 0.025 g 15 g 1 liter
Nutrient agar	Peptone Beef extract Sodium chloride Agar Distilled water	5 g 3 g 5 g 15 g 1 liter
MM	Mannitol Peptone Beef extract Sodium chloride Agar Phenol red Distilled water	2 g 10 g 3 g 5 g 4 g 0.025 g 1 liter
Hektoen	Peptone mixture (including peptones and yeast extract) Lactose Sucrose Salicin Bile salts Sodium chloride Sodium thiosulfate Ferric ammonium citrate Acid fuchsin Bromothymol blue agar	12 g 12 g 12 g 2 g 9 g 5 g 5 g 1.5 g 0.1 g 0.065 g 14 g

Abstract

Salmonella and *E. coli* are among the most common foodborne pathogens causing human illnesses worldwide, with poultry meat being a primary source of contamination. Each stage of the poultry production chain, from farm to table, affects the prevalence of these pathogens. Despite various control strategies to improve the microbial quality of poultry products, complete eradication of these bacteria remains unattained. The objective of this study was to generate new insights into the distribution of Salmonella and *E. coli* throughout different stages of chicken meat production. Additionally, the study aimed to predict the fate of these bacteria across the production chain up to the point just before consumption by examining the effects of varying temperatures and pH levels using predictive modeling tools. A total of 27 samples were collected from the slaughterhouse at five critical steps, and from two butcher shops. More than 16,000 temperature readings and 92 pH measurements were taken during sampling and from cold storage equipment dedicated to chicken meat to monitor the fluctuations of these parameters. Chicken meat was contaminated with Salmonella and *E.coli* at several isothermal conditions (5, 25, 30 and 37°C). Results were then fitted into primary models (Logistic, Gompertz and Baranyi). Measures of goodness-of-fit such as R^2, RMSE and AIc, were used for comparison for these primary models. Based on these criteria, Gompertz model described growth data the best, followed by the Baranyi model. The maximum growth rates obtained from each primary model were then modeled as a function of TºC using the modified Ratkowsky and the gamma concept was used to predict the effects of the T°C and pH on microbial growth. The results of the dynamic analysis indicated that the optimal specific growth rates (μ_{opt})for Salmonella and *E. coli* were 1.63±0.08 1/h and 1.05±0.06 1/h, respectively. Additionally, based on the actual recorded temperatures and pH values, this study found that the populations of Salmonella and E. coli (N_{max}) could reach 8.9 log CFU/g and 8.5 log CFU/g, respectively, after 41 hours, assuming an initial population (N_0) of 1 log CFU/g. Results showed that T°C had a significant effect on bacterial growth, particularly during slaughter when the T°C was close to the (T_{opt}) ($\gamma T = 0.96$). In contrast, pH had a minimal effect on bacterial growth. Understanding these factors can help to act appropriately to prevent contamination. Refrigerating chicken at or below 4°C significantly slows down bacterial growth. Proper cooking effectively kills Salmonella and E. coli, and appropriate cleaning practices can prevent cross-contamination. The results of this study can be used to predict the growth and survival of Salmonella and *E. coli* and assess its risk in chicken or other chicken by-products exposed to a relatively wide temperature range during manufacturing, distribution and preparing.

Key words: E. coli, Salmonella, Primary modeling, Ratkowsky, gamma concept, Temperature, pH.