Prevalence and Molecular Characterization of Some Food-Borne Pathogens Isolated from Chicken Raw Meat

Nareen Abdulaziz Shekhu1*, Jassim Mohammed Abdo2

1Department of Animal Production, College of Agricultural Engineering Sciences, University of D, D, Iraq.

2Department of Biomedical Sciences, College of Medicine, University of Zakho, Zakho, Iraq

*Corresponding author's email: mailto: nareen.shekhu@uod.ac

Email addresses of coauthors: drjassim@uod.ac

Abstract

Poultry meat is a rich nutrient source of microbial growth. This study aims to evaluate and characterize the microbial population in the raw chicken meat. A total of 120 frozen chicken carcasses of twelve different brands were collected from local markets. Additional 120 broiler chicken slaughtered chickens were obtained from local slaughtered shops. The overnight broths were cultured into different specific and selective culture media for each specific bacterium. Morphological method was depended for bacterium confirmation. A single and clean colony for culture was selected and inoculated into specific broth and incubated overnight at 37 ^{o}C. These cultured broths were used in Real-Time PCR detection method. Specific Real-Time kits were used for targeted bacteria following the company guidelines. In the results, *E. coli* was found in 10 out of 36 collected colonies from frozen and imported chicken carcasses while 5 colonies out of 24 were confirmed to be *E. coli* from locally slaughtered chicken carcasses. *Stap. aureus* was detected in 9 colonies out of 22 collected colonies from imported frozen carcasses and 2 out of 7 collected colonies were confirmed as *Staph. aureus* for locally slaughtered chickens. Finally, *Salmonella* was detected in 26 out of 32 tested colonies in the imported frozen chickens and 35 out of 42 colonies were positive to *Salmonella* in the locally slaughtered chickens. DNA samples were amplified for partial 16S rRNA. The amplicons were sent for sequencing in both forward and reverse directions. They were all confirmed as a targeted bacterium and submitted into GenBank through BankIt server. The results of sequencing revealed that most of contaminated chickens were from neighbourhood country (Turkey) with one sample from Ukraine origin. The bacterial infection had a significant effect on meat color and pH. These results conclude that the widespread of bacterial contamination among marketed chicken meat.

Keywords: qPCR, Chicken, Meat, Microbial contamination.

Introduction

Poultry meat serves as crucial dietary protein sources and income in many middle- and low-income countries worldwide. Poultry products' cost-effective and profitable protein production presents significant challenges, particularly due to the widespread use of antibiotics in the poultry industry [8, 18]. The consumption of poultry meat exposure to poultry waste can contribute to the transmission of multidrug-resistant bacteria such as *Escherichia coli*, *Salmonella spp.*, and

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Staphylococcus aureus, which are frequently associated with foodborne illnesses. These bacteria, often found in poultry-derived proteins and by-products, have zoonotic potential, posing a serious threat to public health [10, 23].

The extensive use of antibiotics in poultry farming exerts selection pressure, leading to antimicrobial resistance (AMR) among commensal bacteria such as E. coli, Salmonella spp., and Staphylococcus aureus [17, 24]. These microorganisms, which are naturally present in the human microbiota, can harbor resistance genes or plasmids capable of spreading between humans and animals, thereby exacerbating drug resistance issues [7]. E. coli and Staphylococcus are commensals in the gastrointestinal tract of both humans and animals but can become pathogenic under specific conditions. In poultry, pathogenic E. coli strains are responsible for various diseases, including colibacillosis, meningitis, diarrhoea, and septicaemia, leading to significant economic losses [14]. While most E. coli strains are harmless, approximately 15% are pathogenic, categorized into enterohemorrhagic, enterotoxigenic, enteropathogenic, enteroaggregative, and enter invasive groups based on their mechanisms of disease induction [26]. Additionally, E. coli is classified into commensal, extraintestinal pathogenic, and extraintestinal pathogenic strains based on virulence factors, including toxin production, haemolysis, siderophores, proteases, and adhesins, which play crucial roles in disease development [25].

The culture-based approach is the oldest technology that detects foodborne pathogens in contaminated food. This is a systematic methodology for cultural enrichment that encompasses selective and differential plating, confirmation, and strain characterization [15]. It can be categorized into two types: pre-enrichment, which revitalizes damaged cells, increases the concentration of the target pathogen in food samples, and rehydrates cells from dehydrated food and selective enrichment which utilizes specialized media to augment the concentration of a certain pathogen in food samples [13]. The culture conditions depend on several nutrients in the medium, incubation time, temperature, and air composition [1].

The advancement of molecular biology has greatly enhanced the capacity to detect microbes. Numerous microbiological laboratories continue to employ phenotypic and biochemical techniques for microorganisms' identification. The elevated specificity of molecular techniques facilitates accurately and rapid identification of different cultivated and uncultivated microbial species in specimens, eliminating the necessity for cultivation on nutrient medium in laboratory settings. The substantial advancement of molecular techniques enabled the initiation of a comprehensive investigation of the microbiota and its influence on human wellness in 2007, known as the Global Microbiology Project [21].

The polymerase chain reaction (PCR) technique has established itself as the benchmark in microbiological labs for the identification of microbial organisms. The conventional PCR technique relies on the detection of DNA from bacteria by the amplifying of specific nucleic acid pieces, then followed by sequencing and comparison with databases [30].

Therefore, this study was performed to evaluate the microbial contamination in the frozen imported and locally slaughtered chicken raw meat.

Material and Methods

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Sample Collection

A total of 240 whole chickens' carcass were examined for the detection of salmonella spp., E. coli, and S. aureus. The samples include 120 of twelve different brands of imported and local frozen raw chickens available in supermarkets and 120 of local raw chilled chickens from local slaughter retailers available in Duhok city. The samples were collected and transported to the central laboratory of the College of Agricultural and Engineering Sciences.

Culturing and isolation of bacteria for conventional microbiology

using a graduated cylinder contained water to determine the berries volume, and berries total contents of sugar (%): Taking 1ml from pure juice, add 1ml of phenol 5% then 18ml of distilled water and then 5ml of H2SO4 30 min in boiling water at 60 ∘C The standard sugar solution and standard curve were prepared (Lane and Eynon method) [6]. and total soluble solids (%): The total soluble solids measured witfruits

a 10 g sample and 90 ml of Buffered Peptone Water were mixed thoroughly and enriched in the incubator at 37oC for around 24 h. for salmonella isolation, the pre -enriched culture solution was added to enrichment media, 0.1 ml to 10 ml of Rappaport-Vassiliadis medium (Oxoid, UK), which underwent secondary enrichment for 24 h. The secondary enrichment culture solution was cultured on the selective media of Xylose Lysine Désoxycholate (XLD) agar plates (Liofilchem, Italy) agar incubated at 37oC for 24 h. For E. coli isolation, a loopful from the pre- enriched culture solution was cultured on Eosin Methylene Blue Levine (E.M.B Levine) agar plates (Liofilchem, Italy) and incubated at 37^{o}C for 24+6 h. Regarding staphylococcus aureus identification, a loopful from the pre-enriched culture solution was smeared on Mannitol Salt Agar plates (Liofilchem, Italy) and incubated at 37^{o}C for 24 h. then, a typical colony for each required microorganisms was selected and cultured on selective agar for obtaining a pure culture. The selected colonies were enriched in the Nutrient broth at 37^{o}C for around 24 hrs, then glycerol stocks have been established and stored in freezer at -21 ^{o}C.

Meat colour and pH

The surface colour of chicken breast meat was assessed by utilizing a portable digital colorimeter (WR10QC, FRU®, China) with a 4 mm aperture to investigate Lab values: L* indicating lightness (ranging from 0 for light to 100 for black), a* representing redness and greenness (ranging from 60 for red to -60 for green), and b* denoting yellowness and blueness (ranging from 60 for yellow to -60 for blue). Chicken breast flesh was descaled before applying and testing with a colorimeter.

Pathogen detection via Real-Time PCR

For both Experiment (first and second), the extracted DNA samples were used in Real-Time PCR for specific bacterial diagnosis with the specified diagnostic kits for each studied bacterium. For the E. coli, the E-coli RT-qPCR kit (Bio-Speedy, Turkey) was used. S. aureus RT-qPCR (Bio-Speedy, Turkey) was used for Staphylococcus aureus detection. The Salmonella Genus plus Enteritidis & typhimurium Detection Lyokit (BIOTECON Diagnostics GmbH, Germany) was used for salmonella detection. The reactions were run in Kogene biotech, PowerAmp96 TMDX Real-Time PCR instrument.

Conventional PCR via Partial 16S rRNA amplification

Positive samples from the Real-Time PCR with accepted cyclic threshold (Ct) were chosen for partial 16S rRNA gene

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amplification. A set of primer that was previously published 16S rRNA F-5'-GGAACTGAGACACGGTCCAG -3' 16S rRNA R-5'-CCAGGTAAGGTTCTTCGCGT -3'. A total of 50 µl reaction volume was made containing all the required reaction components using Add Taq master (Add bio, South of Korea).

Phylogenetic tree construction

The sequences of isolated bacterial from meat samples and blast results from NCBI were aligned by using MEGA sequence analyzer Program (version: 11.0.13). Three phylogenetic trees were constructed for each bacterium type by using same program at 1000 bootstraps.

Statistical analysis

For statistical analysis and indicate the significancy among parameters means, SAS statistical analysis software was used (PROG GLM) (SAS 2013). Additionally, Duncan test was run to show any differences among treatment means.

Results and Discussion

The evaluation of meat quality based on color characteristics (L, a, b) and pH values (Table 1) offers crucial insights into the Freshness, processing conditions, and overall acceptability of poultry meat. In this study, significant differences ($P < 0.05$) were observed among broiler chicken samples sourced from local F markets and various imported frozen brands in D province.

Table Local .1 fresh and frozen poultry carcasses L, a, b and pH values

Treatment means	L	a	b	pH
A	53.142^{ab}	0.948^{f}	2.073^{abc}	5.763^{b}
B	52.987^{abc}	1.81^{de}	2.578^{abc}	5.863^{b}
C	51.224^{bcd}	2.131^{bcd}	2.102^{abc}	5.798^{b}
D	52.832^{abc}	2.899^{a}	3.392^{ab}	5.721^{b}
E	51.362^{abcd}	2.651^{abc}	1.461^{c}	6.096^{a}
F	48.655^{ef}	3.044^{a}	1.447^{c}	6.156^{a}
G	53.723^{a}	2.71^{ab}	1.007^{c}	5.732^{b}
H	51.361^{abcd}	2.057^{bcd}	1.736^{bc}	5.679^{b}
I	45.237^{f}	2.656^{abc}	1.416^{c}	6.113^{a}
J	52.139^{abcd}	2.021^{cd}	3.795^{a}	6.142^{a}
K	50.679^{cde}	1.977^{cd}	0.958^{c}	6.124^{a}
L	51.102^{bcd}	2.562^{abc}	2.204^{abc}	5.684^{b}
M	51.041^{bcd}	1.353^{ef}	3.43^{ab}	6.202^{a}
N	50.179^{def}	1.044^{f}	2.26^{abc}	6.29^{a}
O	49.917^{def}	2.187^{bcd}	1.525^{c}	5.873^{b}
P > F	0.0001	0.0001	0.0028	0.0001

a, b, c, d, e, f means in column with different superscripts are significantly different ($P<0.05$).

Figure (1) shows the concentration of E. coli differed significantly among the poultry meat samples. Higher levels were particularly noted in chicken sourced from

local F markets, especially from Local Shop1 and Local Shop2, suggesting possible post-slaughter contamination. These elevated levels could be attributed to poor hygienic conditions during handling, transportation, and retail display. In contrast, some imported frozen brands such as A and I demonstrated relatively low E. coli loads, reflecting better hygienic standards and controlled processing environments.

Moderate E. coli presence was seen in brands like D and L, possibly indicating intermediate hygienic practices. Interestingly, although F chicken meat showed higher pH values (6.156), which generally support microbial growth, the actual contamination level was not the highest, suggesting variability in sanitation and cold chain integrity.

4.2.8 Staphylococcus Concentration

The evaluation of Staphylococcus spp. showed similar patterns, with local F chicken meat exhibiting the highest concentrations (Figure 2). These bacteria, often associated with human contact and poor personal hygiene, were particularly prevalent in samples from Local Shop3 and the D F sample. The high pH and increased a* values (redness) observed in these samples may favor bacterial survival and multiplication.

Imported brands such as J, I, and H exhibited significantly lower Staphylococcus concentrations, indicating better handling and cold storage management. Notably, brands like C and E showed moderate bacterial counts, which could reflect lapses during packaging or distribution phases.

4.2.9 Salmonella Concentration

Among the tested bacteria, Salmonella spp. was of the greatest concern due to their pathogenic potential (Figure 3). Their presence was highest in some local F samples, particularly from Local Shop3, and to a lesser extent in imported brands such as Kand C. These findings point to lapses in hygienic slaughtering conditions and insufficient thermal control.

On the other hand, frozen brands like A and J reported either no detectable Salmonella or very low levels, underscoring the effectiveness of their food safety protocols. Additionally, the low b (yellowness) and moderate pH values in these samples may contribute to reduced bacterial viability.

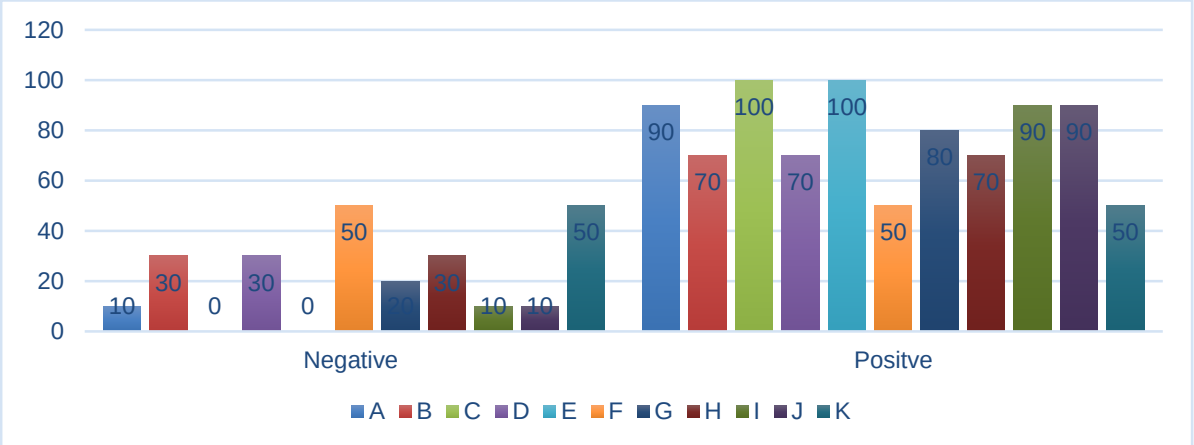

Figure 1: The E. coli concentration in different frozen and F broiler chicken meat in Duhok province

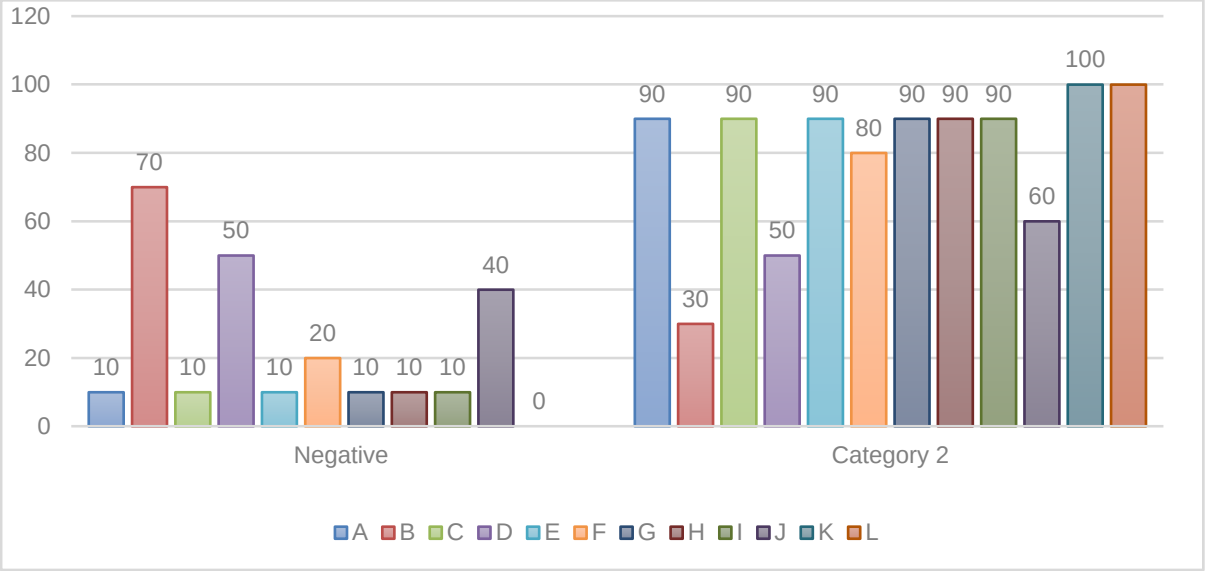

Figure The Staphylococcus concentration in different frozen and F :2 broiler chicken meat in Duhok province

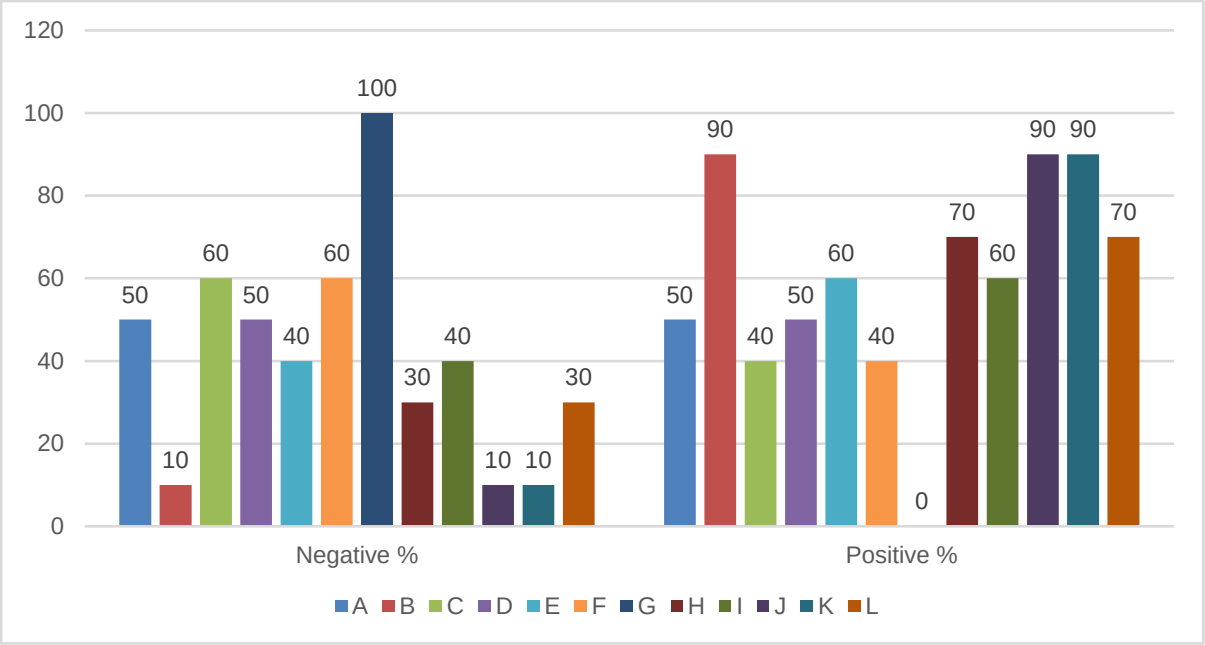

Figure 3: The Salmonella concentration in different frozen and Fresh local broiler chicken meat in Duhok province

Table (2) shows the Real-Time PCR results for the collected samples from frozen chicken carcasses (imported and local brands) and F chilled carcasses from local slaughter markets (Fig. 4-6). For the frozen carcasses, out of 36 collected suspected E. coli colonies, ten colonies were confirmed E. coli. In addition, total of 22 colonies was suspected as staph. aureus morphologically but nine of them

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were confirmed by qPCR. Additionally, 26 colonies were detected positive by qPCR as salmonella from 32 suspected salmonella colonies.

For the locally F carcasses, five collected colonies were detected positive as E. coli from 24 suspected colonies. Moreover, 2 colonies were positive to Staph. aureus out of 7 collected colonies. While out of 42 tested colonies, 35 were detected positive as Salmonella by qPCR.

The positive samples with low Cyclic-threshold (Ct<25) and good amplification were saved for partial 16S RNA amplification by conventional PCR assay

Table 2: Real-Time PCR results for the studied bacterial sample from collected carcasses samples.

Microorganism	Chicken sample types	
	Frozen samples	Local chilled sample
E. coli	10 (36)	5 (24)
Staphylococcus aureus	9 (22)	2 (7)
Salmonella	26 (32)	35 (42)

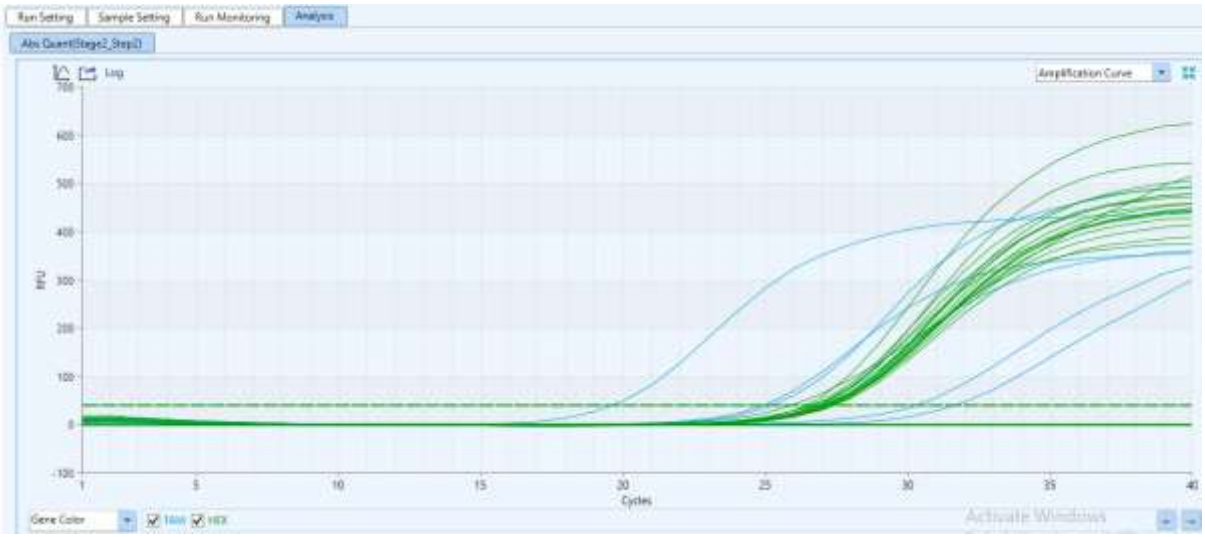

Figure 4: Real-Time PCR amplification curve for E. coli bacterium nucleic acids. Blue line; amplification of targeted DNA (FAM dye) and Green line; Internal control (HEX).

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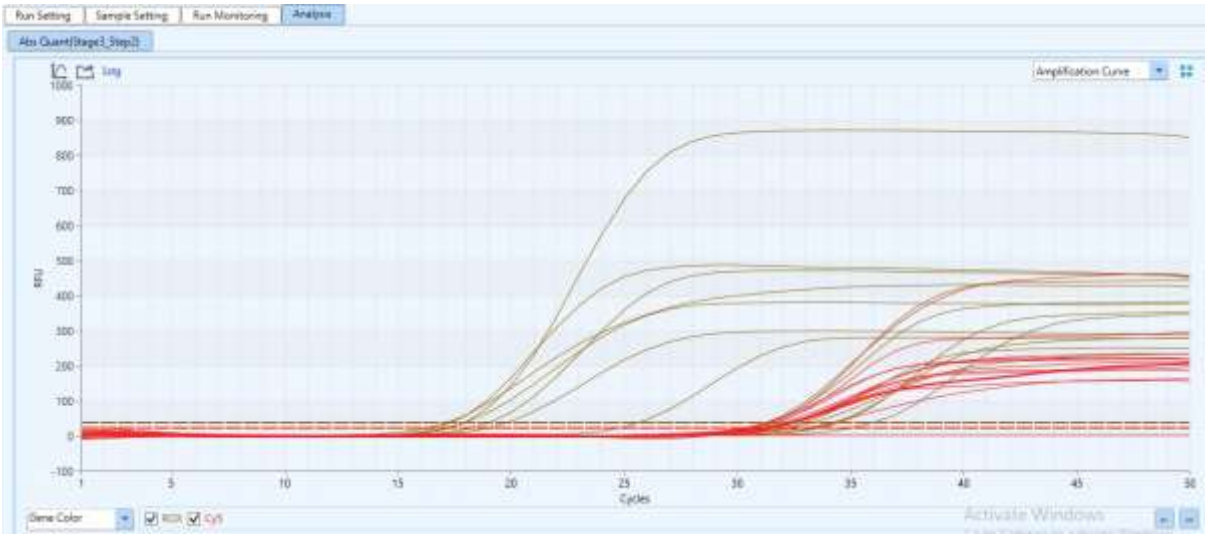

Figure 5: Real-Time PCR amplification curve for Salmonella spp. bacterium nucleic acids. Brown line; amplification of tartgeted DNA (ROX dye) and Red line; Internal control (Cy5).

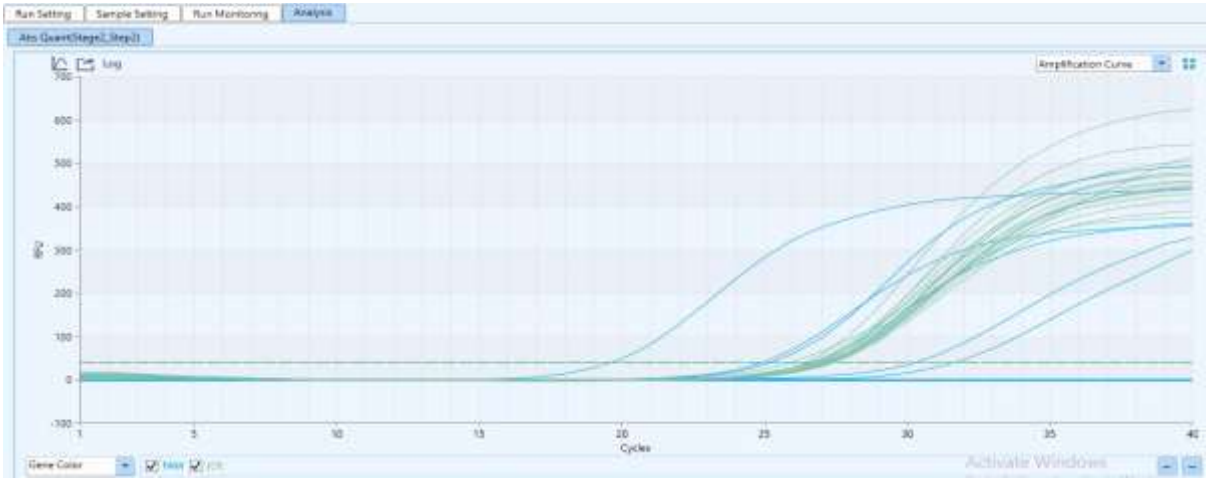

Figure 6: Real-Time PCR amplification curve for Staphylococus aureus bacterium nucleic acids. Blue line; amplification of tartgeted DNA (FAM dye) and Pale Green line; Internal control (JOE).

The qPCR confirmed colonies in the culture media that were collected from studied chicken carcasses (imported and F chilled) were applied to conventional PCR assay by partial 16S RNA amplification using previously published primers. All the tested samples were gained single, good and clean amplification (660 bp) (Figure 7).

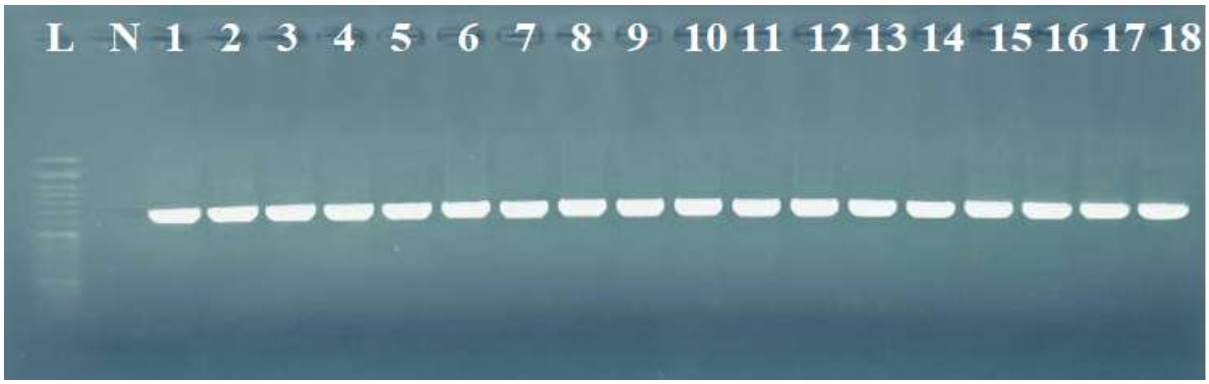

Figure 7: 2% Agarose gel electrophoresis for partial 16S RNA amplification by conventional PCR. Lane L: DNA Ladder (100 bp), Lane N: Negative control, and Lane 1-18: Partial 16S RNA amplicons (660 bp).

The amplified DNA samples (partial 16S RNA, 660 bp) were sent for sequencing to Macrogene company (Seoul, South of Korea). After receiving the DNA sequences (raw data), they were cleaned and aligned. Nucleotide blast results revealed that all colonies that were confirmed by real-Time PCR to each studied pathogens were indicated as specified studied bacterium. After submitting these sequences to GenBank they were each provided with unique accession number E. coli (PV122079, PV200858, PV200861, PV200862, PV200881, PV200906, and PV200907) Salmonella (PV122192, PV123949, PV123951, PV123952, PV123953, PV123954, PV123956, PV147163, PV147165, PV147184, PV147189, PV147192 & PV147225) and Staphylococcus aureus (PV210319, PV210320, PV210321 and OR342420).

Figure (8) shows the phylogenetic tree and genetic relativeness of isolated E. coli from studied chicken meat and some of the blast results from NCBI. At first, the sequence of E. coli isolate (PV122079) that was used for chicken infection experiment had a close genetic distance with E. coli strain; EGI306 (accession no. MN704514) that was isolated Origanum vulgare L. from China. The sequence, PV200907 isolated from imported frozen carcass (origin: Turkey), was clustered in an outgroup branch. Two of the studied isolates, PV200861 and PV200906 that were isolated from challenged broiler chicken in the poultry houses in the Animal Production Department, College of Agricultural Engineering Sciences, University of D, Iraq were clustered as a group and showed the sequence relativeness with PV876997 isolate (strain 11) from Turkey that was isolated from raw milk. The PV200862 (imported frozen carcasses from Turkey) had a strong genetic relativeness with an isolate from China which was identified from a plant. Both isolates PV200862 and PV200858 (imported frozen chicken from Turkey) were in a separate group and the closest isolate was from Italy. The PV200881 was somehow outgroup and rarely had a sequence closeness with other studied sequences.

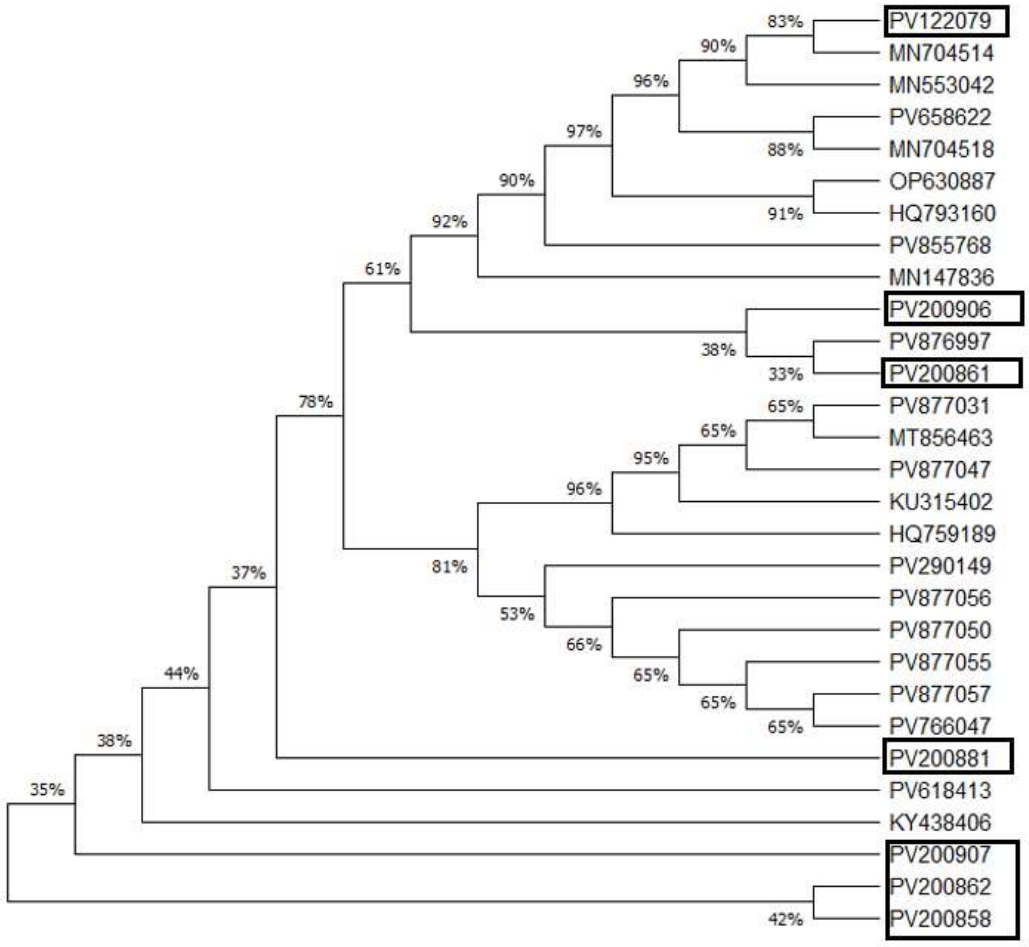

Figure 8: The phylogenetic tree (neibougher joining tree) of the partial 16S rRNA sequence of studied E. coli from raw chicken meat. Black box; the isolated E. coli bacteria and unboxed accession numbers are the blast results from NCBI database. Boostrap analysis (1000 replicates) were used for tree construction in MEGA software.

Figure (9) illustrates the phylogenetic tree and genetic closeness of isolated and studied salmonella partial 16S rRNA sequences. The isolate, PV122192 (that was used in the challenging experiment had a sequence relativeness with salmonella isolates from Iraq and other far distance countries. Overall, all the studied sequences were included in separate clusters. However, one of the sequences (PV147165) that was isolated from locally slaughtering markets was genetically relative to an isolate from Nigeria and identified in a food sample as Salmonella Typhimurium strain 17e.

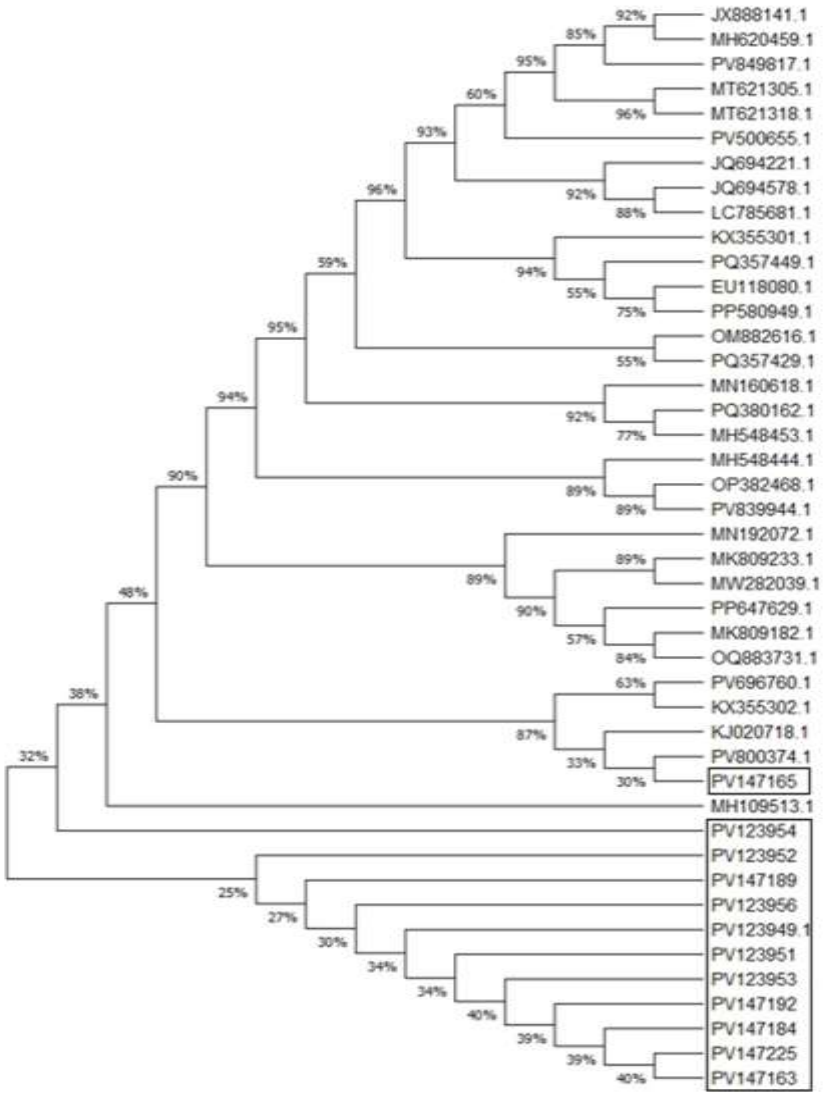

Figure 9: The phylogenetic tree (neibougher joining tree) of the partial 16S rRNA sequence of studied Salmonella spp. from raw chicken meat. Black box; the isolated Salmonella spp. bacteria and unboxed accession numbers are the blast results from NCBI database. Boostrap analysis (1000 replicates) were used for tree construction in MEGA software.

Figure (10) demonstrates the phylogenetic tree of Staph. aureus sequences from chicken carcass from imported frozen and Fly chilled meat from local slaughter houses. The isolate (OR342420) which was in the chicken infection experiment had a genetic closeness with an Egyptian Staph. aureus strain SA. One the studied sequences, PV210320 (imported frozen chicken meat from Turkey), was clustered and had close relativeness with two identified sequences from Saudi Arabia (strain; ABC17) and South Africa (strain; CP019590) isolated from raw milk. Other two studied sequences, PV210319 and PV210321 which were isolated from imported frozen chicken carcasses (origin; Ukraine and Turkey, respectively), were put into a separate group and rarely had a sequence closeness with a Staph. aureus isolate from China.

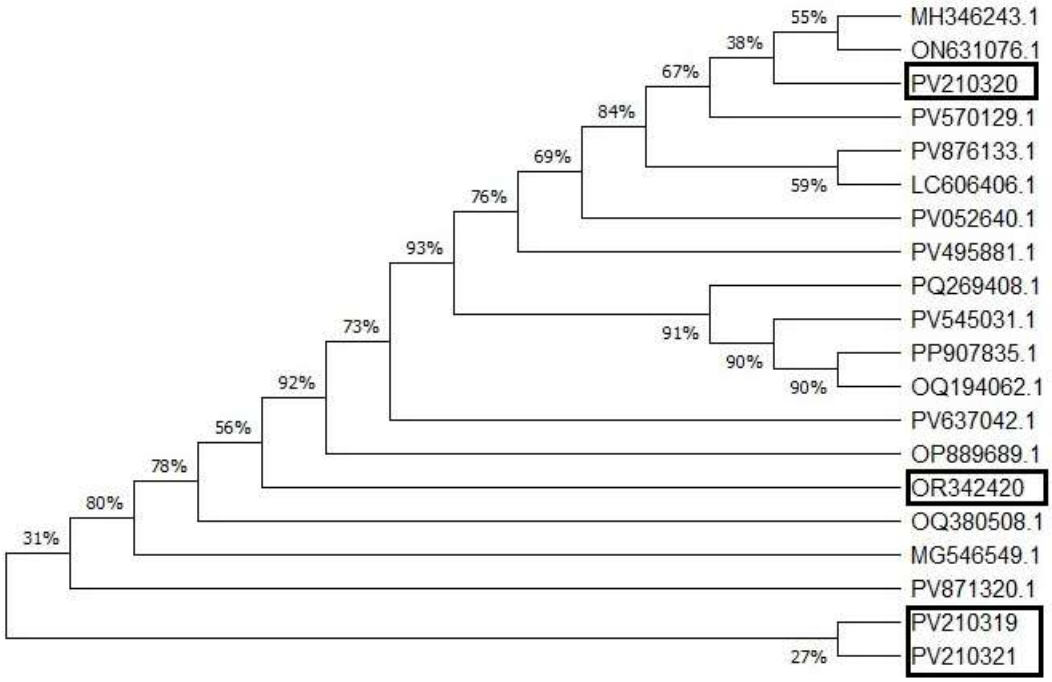

Figure 10: The phylogenetic tree (neibougher joining tree) of the partial 16S rRNA sequence of studied Staphylococcus spp. from raw chicken meat. Black box; the isolated Staphylococcus spp. bacteria and unboxed accession numbers are the blast results from NCBI database. Boostrap analysis (1000 replicates) were used for tree construction in MEGA software.

Discussion

This study provided a comprehensive assessment of meat quality and microbiological safety in broiler carcasses obtained from various sources in D province. The evaluated parameters—color attributes (L, a, b), pH, and microbial concentrations of E. coli, Staphylococcus spp., and Salmonella spp.—demonstrated considerable variability among locally produced F chicken and imported frozen brands. These variations are attributed to differences in slaughtering procedures, handling practices, environmental hygiene, feed composition, and postmortem storage conditions. Below, each finding is interpreted in the context of previous research and underlying mechanisms.

The L value (lightness) is an important visual quality indicator. In this study, imported brands such as G and A showed higher L values, while local F samples recorded lower lightness, indicating darker meat. This aligns with the findings of [31] who reported that rapid chilling and controlled bleeding in industrial settings promote higher L values due to reduced myoglobin oxidation and better moisture retention. On the contrary, darker meat in local samples may be linked to stress-induced depletion of muscle glycogen before slaughter, resulting in limited lactic acid formation and reduced postmortem pH decline—a condition that favors darker color development [6].

The redness parameter (a value) was significantly higher in local F samples such as D and Local Shop1, compared to imported frozen brands. This can be explained by higher myoglobin concentration and delayed postmortem glycolysis, leading to reduced protein denaturation and deeper red coloration [22].

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The b value (yellowness) also varied significantly. Brands such as J and Local Shop1 had higher b values, which may be attributed to dietary inclusion of yellow corn or xanthophyll-rich ingredients. Research by [16] confirms that feed formulation, especially pigment-rich components, directly affects meat color by depositing carotenoids into the muscle tissue.

Meat pH is critical in determining quality, influencing water-holding capacity, color, texture, and microbial stability. The current results indicated that local F broiler meat exhibited higher pH values (up to 6.29 in Local Shop2), whereas imported frozen meat such as A and H, had significantly lower pH readings (around 5.68–5.76). High pH values in local samples can be linked to stress factors such as transport fatigue, long waiting periods before slaughter, and the absence of electrical stunning. These stressors reduce muscle glycogen levels, thereby limiting lactic acid formation and slowing pH decline postmortem [19].

High pH also influences meat color and microbial stability. As demonstrated in this study, higher values (redness) were typically associated with higher pH, a trend supported by [29], who noted that meat with pH>6.0 retains more water and myoglobin, resulting in a darker appearance and favorable conditions for bacterial proliferation.

In the current study, F carcasses from Local Shop1 and Shop2 exhibited the highest E. coli concentrations, likely due to a lack of adequate carcass washing and exposure to contaminated surfaces. Conversely, frozen imported brands such as A and J had minimal E. coli presence, reflecting better processing hygiene and rapid chilling, which slows bacterial multiplication [28].

Staphylococcus spp. were also more abundant in local F meat, particularly from D and Local Shop 3. Since Staphylococcus is commonly found on human skin and mucosa, its high levels suggest contamination during manual handling, possibly by ungloved workers or unsterilized cutting tools. The findings agree with [5], who emphasized the importance of hygiene training for personnel in poultry handling to reduce this form of contamination.

Salmonella spp., known for their pathogenicity, were detected in both local and some imported brands, though the highest loads were again seen in local F meat. According to [11], Salmonella can persist in meat throughout the food chain if proper cooking or storage temperatures are not maintained. The higher detection rates in local meat samples are likely a result of insufficient refrigeration and cross-contamination between raw products during display and sale.

When comparing these findings to similar studies in Iraq, Turkey, and neighboring countries, the results from D are concerning. For instance, a study by [4] reported microbial levels in Iraqi poultry that exceeded international safety limits, particularly in areas with unregulated open markets. Similarly, [12] found that imported frozen chicken had significantly better microbiological profiles due to strict compliance with international safety standards such as HACCP and ISO certifications.

This study confirms that imported frozen brands—processed under standardized hygienic systems—exhibited superior meat quality and lower microbial contamination. On the other hand, the high bacterial counts and elevated pH in local F meat pose a significant food safety concern for consumers in D. Without intervention, these practices may lead to increased incidences of foodborne infections, especially among vulnerable populations.

Prevalence and identification of pathogenic bacteria is challenging in food manufacturing. Different techniques and tools are developed overtime worldwide. Although the conventional microbiological assays are

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efficient and successful, the new era of molecular tools that use the nucleic acid materials are highly dependable. These tools, conventional and Real-Time PCR, are highly sensitive and need small amount of pathogens material to be identified.

In this experiment, both of the techniques were applied and different results were obtained. Based on the molecular technique (Real-Time PCR) more than a half of the conventionally identified colonies from culture media were positive and confirmed by this assay. In the frozen chicken, the most positive colonies were salmonella and local slaughtered chicken with 81.25% followed by the same pathogen from frozen local and imported chicken carcasses being 58.4%. Similar and recent work was done in Baghdad governorate targeting the salmonella detection in locally marketed chicken [2]. This researcher compared conventional technique with PCR and she found that 32% of the collected samples were positive and salmonella genetic materials were detectable. Another research was conducted by [20] and found similar results of salmonella bacterium by conventional PCR. The reason of lower detection in this research compared to our results may be due to the sensitivity and strength of used molecular test in which this author used the conventional PCR technique and the Real-Time PCR was used in our experiment. Another reason may be the number of the samples collected; 120 samples were examined in this research.

Regarding the Staphylococus aureus, lower detection was achieved compared to the salmonella. A 40.90% of the tested samples were positive to staph. aureus in the local chilled and imported frozen chicken. While lowest percentage of staph aureus was obtained in locally marketed and slaughtered chicken (28.57%). Similar work was done by [3] in which she examined a total of 200 raw chicken meat from locally marketed and imported frozen chicken. In the results, she found that 54% of the collected samples were contaminated with Staphylococus aureus by conventional microbiological tests. Additional study was conducted by [9], who examined a total of 100 chicken meat samples for Staphylococus spp. contamnation and found that 31% of the tested samples were contaminated with bacterium. Additionally, both of these experiments used the PCR assay and recommended this technique to be more accurate and sensitive for bacterium detection in meat sample. Furthermore, these results agree with current experimental findings that the bacterium continous contamination occur in raw chicken meat.

In the current experiment, a total of 81.25% and 83.33% of the tested raw chicken meat (frozen imported and locally F chilled meat, respectively) found to be conataminated with Salmonella spp. Comparing to other studied bacterium, the salmonella conamination was almost double or triple in collected raw chicken meat. In a study by [27], they examined a total of 250 chicken meat and they found 8.4% salmonella contamination in the studied samples. However, their results were agree with the present findings in that lower contamination of salmonella was achieved in PCR assay compared to conventional techniques. This may be due to the false positive results in morphological detection of bacterium colonies. Aditional reason is the confirmation of contamination among the studied samples during the testing. In Real-Time PCR, the reliability and positivity of the samples to certain bacterium spp. rely on the several factors such as internal gene amplification and cyclicthreeshold in the amplification curve.

Three separate phylogeentic trees were constructed using the MEGA software including the isolated and sequenced partial 16S rRNA as well as some of the blast hits from the NCBI. While interprating the results, the core point was the most of the studied samples were genetically close to close to the countries which has a direct borders with Iraq. For instance, most of the contamined imported

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raw chicken carcasses with bacterium were from Turkey. This indicates the effect of uncontrolled and weakness of borders in testing these pathogens in the food samples. Additionally, this may be one of the reasons that the locally marketed chicken to get diseased and contamianed with pathogenic bacteria.

Conclusion

This project aimed to evaluate and examine the influence of pathogenic microorganisms (Salmonella, Staphylococcus, and E. coli) on the prevalence of these microbes in marketed chicken meat in the local shop. Overall, the prevalence of pathogenic microbial contaminations in imported frozen and locally slaughtered chicken were examined. Most of the collected samples were identified as contaminated by both conventional microbiological examination and Real-Time PCR assay. These findings conclude that huge bacterial contamination exists among marketed chicken carcasses in local shops.

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