Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 114-128

Evaluation of the Safety Consumption and Adulteration for Red Minced Meat Products in the Local Markets of Baghdad

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Received: February 20, 2025 / Accepted: May 8, 2025 / Published: November 16, 2025

Abstract: Meat serves as a rich source of numerous vital nutrients for humans, the contamination, adulteration and fraudulent of minced meat have raised a serious legal, religious, and medical problems in Iraq and other countries. The purpose of this study was to investigate the minced meat validity throughout detecting of common bacterial contamination and identifying animal meat species in addition to investigate of halal authentication using conventional and molecular methods. A total of (100) distinct minced meat items were randomly gathered in various regions of Baghdad city. The first step in this study was explore the bacterial contamination with routine and molecular assays, meat tissue were cultured on different media. So, bacterial isolates were subjected to macroscopical, microscopical and biochemical tests, for more confirmation the molecular diagnosis using *16SrRNA* as a housekeeping-genes was conducted by PCR. Also, four sets of primers were designed to test the pathogenicity of bacterial isolates by targeting of virulent gene for each one as *gap, ttr* and *rfb* genes *for Staphylococcus aureus, Salmonella spp. and Escherichia coli O157:H7* respectively. The second step of focused on animal meat species, by molecular assay highlighted the origin of meat species, a set of eight primers specific to *cytochrome (cyt) b* gene family for detect the chicken, cattle, sheep, goat, horse, pig, donkey and dog meat. One hundred minced meat samples examined revealed that there was a significant level of bacterial contamination involved 43%, 9% and 9% by *Staphylococcus aureus, Salmonella spp*. and *E. coliO157:H7* respectively. And, 24% adulteration and mislabeling in beef minced meat label with sheep, goat, and chicken. Tow positive sample were recorded for donkey species, while negative results were documented for dog, horse and pig meat species in all examined sample. The study determined that the examined meat products showed presence of a high percentage of bacterial contamination in meat products, which poses a direct threat and danger to human health. Clear evidence of adulteration. The employed mitochondrial DNA based multiplex PCR proved to be a valuable and uncomplicated technique for verifying the species of the meat products.

Keywords: Meat products, Meat contamination, Multiplex PCR, Meat adulteration.

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Introduction

Meat is defined as the flesh of animals used as food, meat embodies a good source of many essential nutrients for human beings. It is abundant in protein, essential amino acids, lipids, vitamins, minerals, and other nutritional components (1). Day by day dramatic growth in total world population has increased, reach about 8.1 billion (2), that increased the demand for meat globally, so in the last years, adulterators have been elevated too (3 Numerous issues have arisen as a result,

particularly in light of the ongoing reports of adulterants in meat products that endanger their quality and safety(4).

Adulteration in meat products refers to the fraudulent deliberate which take two lines, the first line includes microbial meat contamination (5). Various pathogenic bacteria, including *S. aureus, Salmonella spp. and E. coli* can be found in meat. Therefore, meat poses a serious risk to human health since it can quickly result in food-borne illnesses and inadequate controls against those microorganisms, The second line include addition or substitution completely or partially one of meat species or more than with a cheaper alternative type or tissues can be easily hidden (6). In Iraq and according to ministry of health and health control department, many cases of food poisoning from contaminated meat with bacteria have been recorded in many hospitals and health centers (7). The adulteration in a local and imported meat which were seized through inspection campaigns at the borders, local markets and restaurants and had a significant negative effect on the Iraqi consumers and posing a great danger to people's lives (8).

Detecting of adulteration in meat is crucial for identifying and preventing many complicate related to health and safety. False or deliberate mislabeling of meat products, which cannot be easily identified using traditional methods, is still predominant and widespread in Iraq and global (9, 10).

The study aimed to investigate bacterial contamination in minced through routine and molecular examination in some Iraqi local markets and identifications of meat species by using conventional and multiplex PCR for eight suspected animals' species (chicken, cattle, sheep, goat, horse, donkey, dog and pig with detection of halal authentication meat.

Materials and Methods

Samples collection

A total of 100 minced meat samples were collected from various sources, including butchers, supermarkets, and street hawker carts in the Baghdad area. The collection period spanned from June 2023 to June 2024.

Bacterial sample preparation for inspection

A 25 grams were taken from each specimen and combined with 225 milliliters of 0.1% sterile peptone water. The mixture was stirred for 2-4 minutes and then permitted to stand for around 5 minutes at room temperature. Following this, 10 serial dilutions were performed to count the bacteria under completely sterile conditions as describe by (11).

Determination of total *Staphylococcus aureus, Escherichia coli, and Salmonella spp.* Counts

The quantification of *S. aureus, E. coli, and Salmonella spp*. was conducted by transferring 1 ml from each of the pre-prepared serial dilutions onto plates containing (Baird-Parker Agar, Xylose Lysine Deoxycholate Agar, Sorbitol MacConkey Agar, Eosin methylene blue, Mannitol salt, and Salmonella Shigella agar). The plates were then incubated at a temperature of 37°C for a period of 24 hours. The colonies were enumerated and subsequently diagnosed using the API 20E kit following the manufacturer's instructions to determine the biochemical characteristics of the isolated microorganisms (11).

Identification by serology

By employing the slide agglutination technique as delineated by(12), the somatic (O) antigen of *E. coli* was identified. Flagellar (H) antigen serotyping was conducted in accordance

with the methodology outlined by (12). Purchased *Salmonella spp.* serotyped anti-O-sera were those described by (13).

Molecular bacterial diagnosis

The three sets of primers of this study were designed to anneal the regions of the targeted genes, to confirm specificity, and to permit species-specific detection. Identification of *Staphylococcus aureus, Salmonella spp. and Escherichia coli O157:H7.* at the molecular level was performed by partial amplification of the *16S rRNA* nuclease for each one. Amplicon sizes of determined genes regions are used for the primers design are mentioned in Table (1).

Table (1): Primer's sequence of *16S rRNA* used for *Staphylococcus aureus, Salmonella spp. and Escherichia coli O157: H7* detection with their amplificon size and annealing.

Primer	Sequence (5'-3'direction)	Amplificon size
staphylococcus spp.	5'-AAGGTCTTCGGATCGTAAAAC-3 5'-GCACTCAAGTTTTCCAGTTTC-3'	246bp
salmonella spp.	5'-AGCAAACAGGATTAGATACCC-3' 5'-TAACCCAACATTTCACAACAC-3'	316bp
- E. coli	5'-TGATCATGGCTCAGATTGAAC -3' CCACCTACTAGCTAATCCCAT-3'	245bp

This study: The primers were designed by using the NCBI/Primer3 program

The other three sets of primers were designed to confirm the bacterial isolation for each of *Staphylococcus aureus, Salmonella spp.* and *Escherichia coli O157: H7,* by targeting of virulent genes as glyceraldehyde-3-phosphate dehydrogenase (*gap*), and tetrathionate reductase (*ttr*) genes and cluster (*rfb*) gene respectively as mentioned in Table (2).

Table (2): Primer's sequence of some virulent genes used for *Staphylococcus aureus, Salmonella spp. and Escherichia coli O157: H7* detection with their amplificon size and annealing temperature.

Primer	Sequence (5'-3'direction)	Amplificon size
gap gene staphylococcus aureus,.	5'-GCGGTTATTATACGACGATGT -3 5'-TAACACTGTATTCTTGGGTGC-3'	392
ttr gene salmonella spp.	5'-AGCTGGACATGGTATTTGATT-3' 5'-GTCTCAATGGAAGCATTTTGT-3'	532
rfb gene E. coli. O157: H7	5'-ATTCTAAAGGAGGTACAGCCA-3' 5'-CAAACATGATTCCAAGCCTTG-3'	418

This study: The primers were designed by using the NCBI/Primer3

The DNA Extraction from Bacteria and dsDNA Quantitation by Qubit 4.0 were done and PCR reaction mixture and setup as in Table (3 and 4).

Table (3): Reaction mixture of PCR working solution.

Component	Reaction volume (µl)
Forward primer	1
Reverse primer	1
Template DNA	2
Nuclease free dH2O	8.5
Master Mix,2X	12.5
Total volume reaction	25

Table (4): The PCR condition for bacterial genes detection.

Step	Temperature (℃)	Time	No. of cycles
Initial denaturation	94	5 min	1 cycle
Denaturation	94		
		30 sec	30x
Annealing	53	45 sec	
Extension	72	45 sec	
Final extension	72	7 min	1cycle

DNA extraction from meat samples

Meat sample (25 mg) which was placed in a 2 ml micro centrifuge tube. Extractions were achieved following the Dneasy Blood and Tissue kit manufacturers protocol (Promega, USA).

Detection of Meat Species Adulteration

- **Primers of *Cytochrome b* gene:** A set of six primers specific to *cyt b gene* family as mentioned by (14) was custom synthesized at Cinagen to detect the Goat, Chicken, Cattle, Sheep, Pig and Horse meat pol 1 (P1) as described in Table (5).

Other two set of primers specific to *cyt b gene* family was custom synthesized at Cinagen to detect the Donkey and Dog meat as pol 2 (P2) as shown in Table (6).

Table (5): *cyt b* gene primers sequences of animal meat species of Goat, Chicken, Cattle, Sheep, Pig and Horse. pol 1 (P1).

Name	Primer	Sequences (5' – 3')	No. of Bases	Reference
Common	F	5'GACCTCCCAGCTCCATCAAACATCTCATCTTG ATGAAA-3'	38bp	(14)
GOAT	R	5'-CTCGACAAATGTGAGTTACAGAGGGA-3'	26bp	
CHICKEN	R	5'-AAGATACAGATGAAGAAGAATGAGGCG-3'	27bp	
CATTLE	R	5'-CTAGAAAAGTGTAAGACCCGTAATATAAG-3'	29bp	
SHEEP	R	5'-CTATGAATGCTGTGGCTATTGTCGCA-3'	26bp	
PIG	R	5'-GCTGATAGTAGATTTGTGATGACCGTA-3'	27bp	
HORSE	R	5'-CTCAGATTCACTCGACGAGGGTAGTA-3'	28bp	

Table (6): The *cyt b* gene primers sequences of animal meat species of Donkey and Dog. pol 2 (P2).

DONKEY	F	5-TACTACGCTCGTCGAATGA-3	19bp	This study
	R	5-AAGGATAAGGGCTAATACACC-3	21bp	
DOG	F	5'-AACTGACTTAGTAGAATGGATCT-3'	23bp	
	R	5-AAGTGAGAAGATCGGCGA-3	18bp	

- **Reaction set up**: Conventional PCR was performed in a 25 µl total volume, as described in Table (7).

Table (7): Reaction setup for conventional PCR.

Component	Reaction volume(µl)
Master mix	**12.5**
10X primer forward	**1**
10X primer reverse	**1**
Template DNA	**2**
RNase –free water	**8.5**
Total reaction volume	**25**

Cycling conditions

Cycling parameters for conventional PCR are presented in Table (8).

Table (8): Cycling conditions for conventional PCR.

Step	Temperature (°C)		Time	No. of cycles
Initial denaturation	95		3min	1 cycle
Denaturation	94		30 sec	35 cycles
Annealing	**P 1**	**P 2**	30 sec	
	62	55		
Extension	72		30 sec	
Final extension	72		10min	1cycle

PCR Assay

The previously designed simplex and multiplex assay was confirmed for its specificity through cross-amplification with several meat species. The amplification protocol included an initial denaturation, denaturation, annealing and extension.

A 50 µl reaction mixture was assembled in an Eppendorf tube, consisting of 25 µl of master mix, 15 µl of 10X primer mix, 7 µl of RNase-free water, and 3 µl of target DNA (comprising all species). Subsequently, 2 µL of PCR results were combined with 2 µL of gel loading solution and placed onto a 1.5% agarose gel. The gel was then subjected to an electric field of 50 V for a duration of 1 hour, specifically targeting a 10-µl segment of the amplified DNA fragments. The gel obtained was treated with ethidium bromide (0.5 µg/ml), seen under a UV transilluminator, and captured using a Polaroid camera.

Table (9): The cycling conditions used for multiplex PCR.

Steps	Temperature	Time	No. of cycles
Denaturation 1	95°C	5 min.	1 cycle
Denaturation 2	94°C	30 sec.	35 cycles
Annealing	62°C	90 sec.	
Extension	72°C	90 sec.	
Final extension	72°C	10 min.	1 cycle

Results and discussion

Detection of *Staphylococcus aureus*

In present study clear growth observed on the surface of Baird-Parker agar (BPA) medium which used as a selective method to identify *S. aureus*, and the culture feature appeared on surface of this medium after 24-48 hrs. when incubated at 37 Cº as black colonies which are encircled by a whitish halo zone as described by (15).

According to the above mentioned features, 43% of samples which obtained from minced were positive for *S. aureus*, with high count mean value 3.4×10^5 beside it was found 8 out of 43 (18.6%) of minced meat samples unfit for human conception due to its contain more than 1×10^5 CFU as shown in Table (10).

Table (10): Incidence and count of *Staphylococcus aureus* in minced meat samples.

Bacteria	No of samples	Positive sample		*Staphylococcus aureus* count mean value	No. of unfit for human conception	
		No +V	%		No	%
Staphylococcus aureus	100	43	43	3.4×10^5	8	18.6
Salmonella spp	100	9	9	-	9	9
E. coli O157:H7	100	9	9	-	9	9
P-value	----	---	0.0001 **	0.0001 **	---	0.0001 **
** (P≤0.01).						

The high contamination in minced samples may be got from tools, machinery, equipment like hooks, knifes, tables, cutting boards and mince meats machines etc. that needed to processed these products and which plays a major role in contaminating meat with bacteria, especially when the equipment is not daily washed with water and detergents with basis program, this findings support by other Iraqi researchers as Ahmed *et al*. (16) who observed high prevalence of bacterial contamination including *S. aureus* in minced meat (84%), beef meat (48%) and chicken meat (44%) in Dohok city. Similar obtain by Abdul Qader and AlKhafaji (17) when found high isolation rates of bacteria in imported and local red meat in Baghdad city.

These results agreed with Egyptian study by Karmi (18) which conducted on 200 meat products samples and noticed that, the prevalence of *S. aureus* was 20%, 24%, in minced meat and luncheon respectively.

Molecular identification of *S. aureus*

The present findings recorded 43 out of 100 (43%) samples were positive reaction to *16SrRNA* (Figure 1).

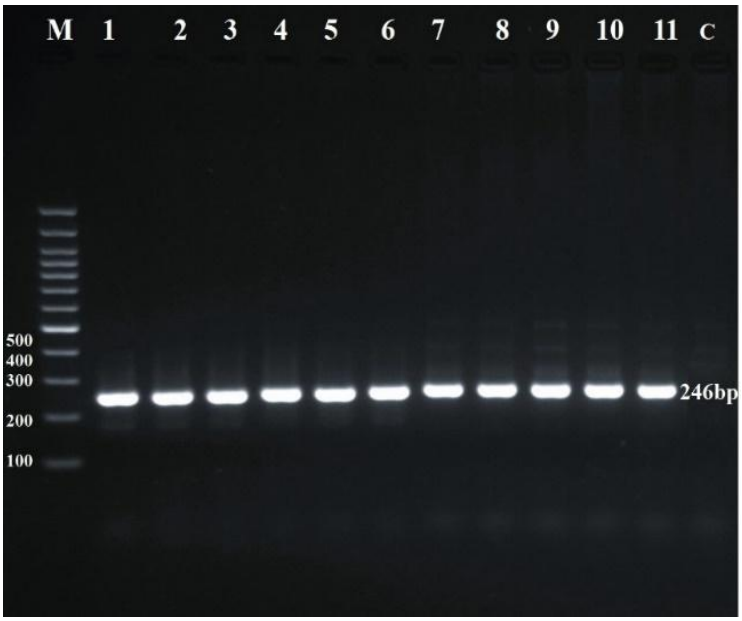

Figure (1): Agarose gel (1.5%) stained by RedSafe® and 80V electrophoresis for *16S rRNA* gene. Lane 1,2,3,4,5,9,10 and 11 shows PCR product of *16S rRNA* gene with an expected size of 246 bp. M: DNA ladder (100 bp step), -C: Negative control

The rate of PCR positive indicates a highly conserve sequence design of this *16S rRNA* gene of *S. aureus*, A strong correlation was seen between the PCR methodology and the culturing method, as positive samples yielded positive amplification results using conventional PCR.

Multiplex PCR technique also used in the differentiation of the *16SrRNA* and *gap* genes, and there was high degree of specificity in DNA bands (Figure 2). This was due to the reason that the other bacteria weren't showing any specific amplification when examined with gel electrophoresis and a UV eliminator.

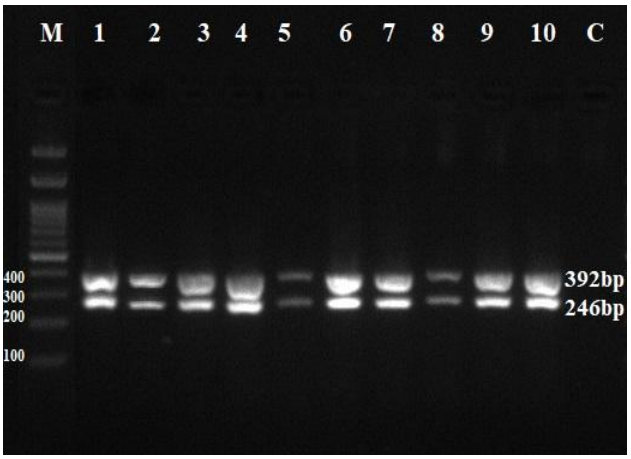

Figure (2): Agarose gel (1.5%) stained by Ethidium bromide dye and 80V electrophoresis for *16S rRNA* and *gap genes* (Multiplex PCR) with an expected size of 246bp and 392bp, respectively. Lane 1,2,3,4,5,6,7,8,9 and 10 positive for both genes. M: DNA ladder (100bp step), -C: Negative control.

The outcome showed highly expression in most of isolates, this reveled that the *S. aureus* isolates have high pathogenicity that form dangerous on public health, although only a few numbers of samples exhibited negative amplification.

The PCR has been successfully employed in many Iraqi researches to identify clinically significant microorganisms. Previous studies have focused on comparing the pathogenesis of clinical and environmental microorganisms. For instance (19) investigated the distribution of important food pathogens, such as *S. aureus*, in raw beef and lamb meat using multiplex PCR targeting the *uec* gene. His study demonstrated that multiplex PCR is a valuable tool for detecting food-borne pathogens, especially *S. aureus.*

Ali *et al*. (20) conducted a study in Syria where they used PCR to amplify the sequences of *16S rRNA*, *gap* gene, and *nuc* gene using six unique primers. The purpose of their study was to explore the presence of *S. aureus* in clinical samples, and the results demonstrated that PCR was a reliable and widely accepted method for identifying and classifying *S. aureus.*

Detection of *salmonella spp.*

The colony on Xylose Lysine Deoxycholate (XLD) agar appeared as round pale colonies with a black center. The outcome of biochemical tests confirmed the isolates identification. The API 20E were also utilized in the diagnosis process for additional conformation.

According to the diagnostic features 9 (9%) *Salmonella spp.* positive samples were obtained from minced as shown in Table (10). All these samples represent unfit for human conception according to the criteria of Iraqi central organization for standardization and quality control which, consider the presence of even one *Salmonella bacteria* in a meat sample is unacceptable and rejected sample.

The high number of isolates in minced meat may be attributed to that, this meat was prepared completely or partially from imported meat, which have a great chance of *salmonella* growth compared to local meat. The export operations from origin to destination, long shipping period and poor storage by freezing and thawing give a good opportunity for the growth of *salmonella*. The results came complied with other study in Iraq done

by AL-mossawei *et al.* (21) which recorded a significant elevation in *salmonella spp*. isolates from Indian and Brazilian imported meat.

Many studies conducted to detect the *Salmonella spp.* in meat and meat products and obtain various results as AL-mossawei *et al.* (21) who isolate 11.9% in imported meat and Karmi (18) who able to identify *Salmonellae* in luncheon at 10%, and which was less than present results, in contrast Ahmed *et al.* (16) observed high contamination with *Salmonella spp*. isolates reached to 65%in chicken meat, and Shaltout *et al.* (6) who were unable to find *Salmonella spp.* in the luncheon.

Molecular identification of *Salmonella spp.*

The results recorded that all isolates gave positive (100%) for the presence of *16SrRNA* through appearing of (316bp) fragment of this gene (Figure 3).

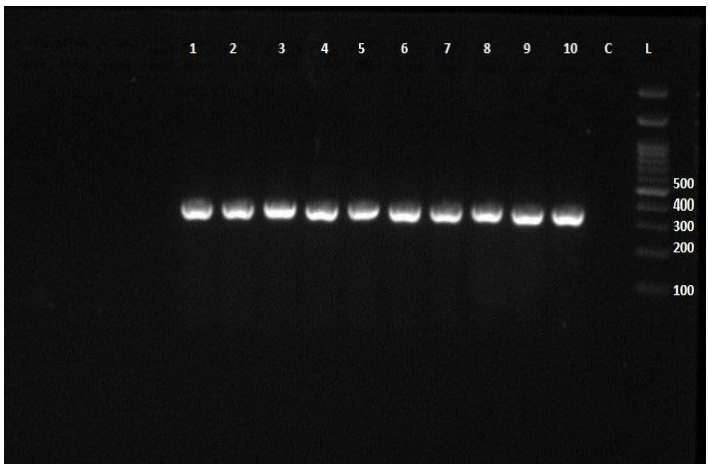

Figure (3): Agarose gel (1.5%) stained by Ethidium bromide and 80V electrophoresis for *16S rRNA* gene. Lane 1,2,3,4,5,6,7,8,9 and 10 shows PCR product of *16S rRNA* gene with an expected size of 316 bp. L: DNA ladder (100 bp step), -C: Negative control.

Regarding to the *ttr* gene, the current results reveled 8 out of the 9 isolates (88.8%) were positive for the *ttr* gene (532bp). All primer sets used in the experiment successfully produced PCR products of the estimated sizes for *Salmonella spp.* with equal levels of amplification (Figure 4).

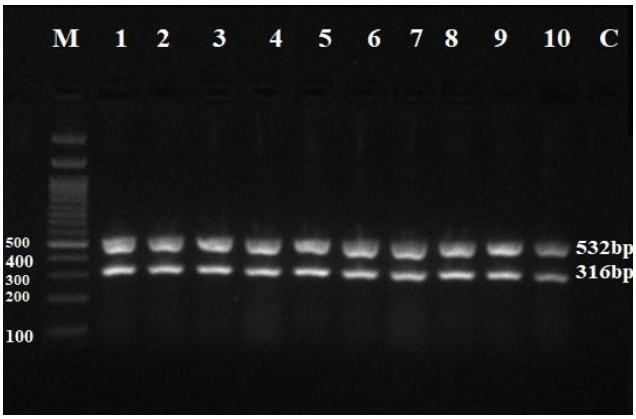

Figure (4): Agarose gel (1.5%) stained by Ethidium bromide dye and 80V electrophoresis for *16S rRNA* and *ttr* genes of *Salmonella spp.(*Multiplex PCR) with an expected size of 316bp and 532bp, respectively. Lane 1,2,3,4,5,6,7,8,9 and 10 positives for both *16S rRNA* gene and *ttr* genes only. L: DNA ladder (100bp step), -C: Negative control.

Many Iraqi studies have reported similar findings such as Ahmed and Khudor (22) who conducted a study in Basrah governorate where they noticed *Salmonella* isolates in 205 frozen and fresh chicken in addition to beef meat samples, using standard biochemical test and API 20 E system had an identification accuracy of 76.0% and 84%, respectively, while the PCR technique using *16srRNA* to achieve a perfect identification rate of 100% (20 out of 20 samples). Nowadays, recently similar molecular technique were used to *Salmonella* detection by other Iraqi researchers as Abdulrahman (23) who depended on multiplex PCR to isolated *Salmonella enteritidis* and *salmonella typhimurium* in raw chicken meat. Another work by Al-Shafee and Abdulwahid (24) used PCR assay and sequencing techniques to analyze three virulence factors genes (*stn, avr A, and sop B*) of *Salmonella spp.* in a total of 300 chicken product samples in Wasit province.

Detection of *E. coli O157:H7*

The bacteria were identified using appropriate culture media and biochemical tests, they were cultured on Sorbitol MacConkey agar (SMAC), which is a type of media that helps differentiate Entero heamorrgic *E. coli* (EHEC) from other serotypes of *E. coli* by using 1% D-sorbitol instead of lactose. Pale colonies were observed on the SMAC, indicating that the bacteria in question were *E. coli O157 H7*.

Also, for more confirmation the serological test were done, through a slide agglutination test with antisera O157 and H7 which gives a positive reaction to agglutination according to Koneman *et al.,* (25).

The current result found 9 out of 100 (9%) were *E. coli O157: H7* isolates. This strain is one of the important standards in evaluating the suitability of meat for consumption according to the Iraqi organization for standardization and quality control because it is considered one of the important pathotype bacteria.

The current results are similar to those of Ibrahim *et al.* (26) who recorded that the *E. coli* O157 isolated in meat products at a greater rate and more than that in milk 73% and 26% respectively, the total of bacterial isolate were 30 (10%) from 300 sample in Baghdad governorate. Also, Al-Chalaby (27) who shows that the highest percentage of the pathotypes *E. coli* (EHEC and ETEC) are in minced meat from restaurants and butcher shops, 40% and 46.7%, respectively.

Molecular identification of *E. coli*

Among the all tested meat product samples in this study, 9 isolates were confirmed as *E. coli* by PCR assay using the *16SrRNA* gene (245bp) specificity for all *E. coli* isolates, that indicate a highly conserve ribosomal RNA sequences design choosing (Figure 5).

The multiplex PCR technique is an appropriate method for quickly identifying *E. coli O157:H7* at the species level. This method has the potential to expedite and streamline the identification process, allowing it to be finished within a single working day.

The results indicated 8 out of 9 tested sample were positive for this gene for *E. coli O157:H7* detection.

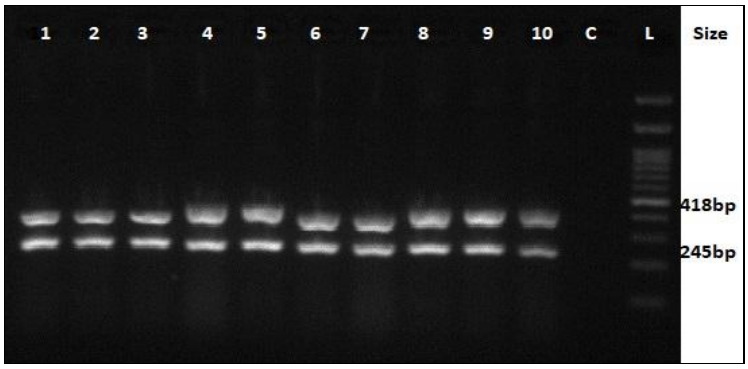

Figure (5): Agarose gel (1.5%) stained by Ethidium bromide dye and 80V electrophoresis for *16S rRNA* and *rfb* genes (Multiplex PCR) with an expected size of 245bp and 418bp, respectively. Lane 1,2,3,4,5,6,7,8,9 and 10 positives for both *16S rRNA* gene and *rfb* genes only. L: DNA ladder (100bp step), -C: Negative control

Several researches have confirmed that multiplex PCR is a dependable approach for identifying *E. coli O157:H7* and using suitable genes for final detection. In local study Jwied and Jebur (28) used PCR to find shiga toxin genes in *E. coli* isolates throw *rfb* gene which encodes the O-antigen and the *stx* genes, their results showed a significant degree of similarity in their sequencing identity of *E. coli* strain (O157:H7) isolates from humans and animals, as determined by NCBI-Blast.

Rani *et al*. (29) provide a quick, easy, sensitive, specific, and portable way to identify *E. Coli O157:H7* in food and assessed the impact of gene selection in identifying *E. Coli O157:H7* by combining a polymerase amplification with *rfb*, *fliC*, and *stx* gene targets and other tests.

Meat products analysis for species detection

The one hundred red meat products analysis, with label of manufacturer as beef. The results showed all collected samples (beef label) were positive for cattle (100%), this is evidence that there is no any product in the Iraqi market that is completely adulterated but its recorded only partial fraudulent with other meat species.

Depending to type of meat products, 24 out of 100 (24%) minced samples showed a percentage of mixing with another undeclared species as shown in Table (11), which distributed as 24/24 (100%), 2/24 (8.3%) and 9/24 (37.5%) in sheep, chicken and goat respectively (Figure 6).

Table (11): Analysis of the commercial beef red meat products for authentication.

Red meat products	No of samples	Label of manufacturer	No of positive samples	Percentage of positive samples	beef identification species	Ingredient adulteration with fraud ratio						
						sheep	chicken	Goat	horse	donkey	dog	pig
Minced	100	Beef	24	24%	+	+ 24/24 100%	+ 12/24 50%	+ 9/24 37.5%	- 0/24 0%	- 2/24 8.3%	- 0/24 0%	- 0/24 0%
P-value	--	--	--	0.0001 **	--	0.0001 **	0.0001 **	0.0001 **	NS	0.298 NS	NS	NS

*** Significant (P≤0.05). ** Highly Significant (P≤0.01). NS = None significant.**

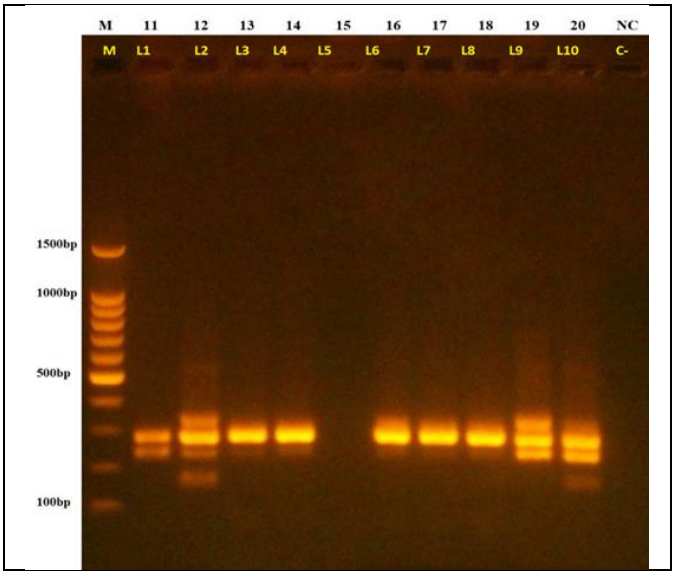

Figure (6): Agarose gel (1.5%) stained by Ethidium bromide dye and 80V electrophoresis for different PCR products representing red meat labeled production Lane 11: chicken (227bp) and cattle (274bp), Lane 12: 4 species mixture, Lane 13, 14, 16, 17, 18 cattle, Lane 19, 20 mixtures, M100 :100 bp ladder.

The current results exhibit high percentage of red meat adulteration, the analyzed processed meat products were found to be vulnerable to deceptive adulteration for the purpose of financial advantage by combining them with less expensive meats such as poultry flesh as a partial substitute due to high prices of the local red meat comparison with white meat.

The high adulteration percentage of red and white meat in most Iraqi processed meat may be due to using the local animal fat of cattle especially tail-fat of sheep which characterized by good flavor and desirable in Iraqi people.

The significant increase in meat prices in Iraq which reached and recorded a high level during last years has greatly contributed in the increase cases of fraud and deception, and the continuation of this states will lead to elevation of fraud in the local markets.

These finding were agreed with Hossain *et al.* (30) who identified a significant prevalence of adulteration in Bangladeshi beef-labeled products with an estimated 32% mislabeling rate, this may be attributed to the higher economic value of beef flesh products of foreign origin were corrupted with mislabeled with buffalo flesh. Also, the current appearance aligns with previous worldwide studies about the adulteration of chicken and equine meat in oriental sausage samples, as well as beef luncheon. However, the percentages of adulteration may vary between countries in beef luncheon and beef burger(31).

The current finding was conflicted with another investigation. In Egypt study conducted by Abuelnaga *et al.* (32) discovered that 88.5% (124 out of 140) of the tested meat samples were positive for chicken, the research revealed a significant prevalence of species adulteration, especially, all minced beef meat and beef burgers and 95% of kofta included chicken replacement.

Also, in Egypt -Suez Canal cities (33) reported that beef meat was not discovered in 20% of the samples while other ten percent contained equine species. In addition, beef burger meat products were adulterated with equine

species at a rate of 30% and mislabeled with poultry meat species in all sample.

The present investigation documented notable findings when it revealed two samples at some street vendors that tested positive for donkey meat 2/24 (8.3%) in the eastern Baghdad region, exactly in the Al-Ubaidi district. This state may be attributed to, these products prepared either at home or in unlicensed small factories and distributed to street vendors at a cheap price. Also, the minced meat are usually made by mixing high proportions of spices and flavors in addition to using different sauces that may encourage cheating and make the taste palatable and acceptable in an unknown way. The obtained data were less than that in Egyptian study by Omran *et al.* (34) who recorded high significant percentage 20% of donkey meat mixed with burgers and 20% with kofta (minced of kebab) product.

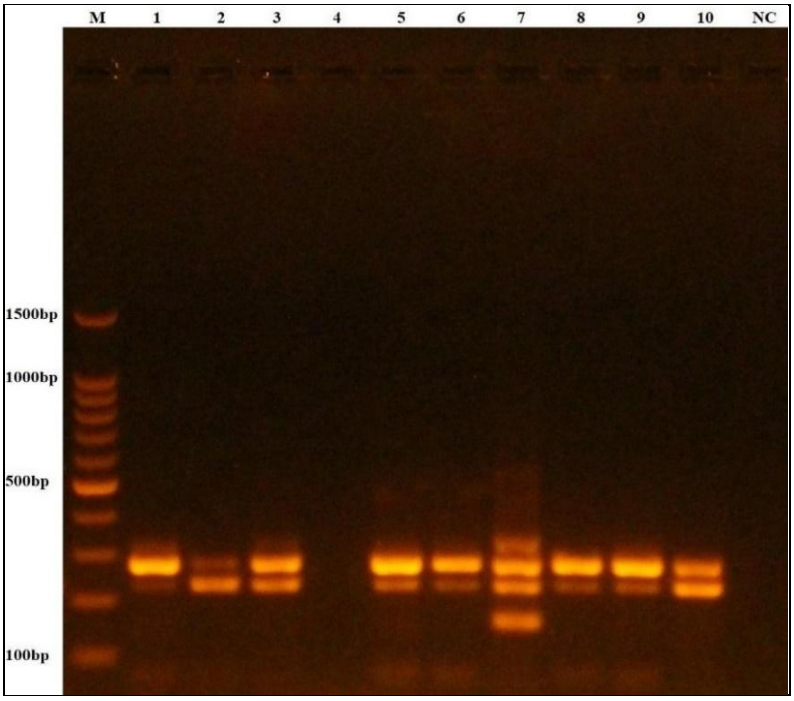

Figure (4-25): Agarose gel (1.5%) stained by Ethidium bromide dye and 80V electrophoresis for different PCR products representing red meat labeled production Lane 1,2,3,5,6,7,8,9,10 : cattle (274bp), Lane 3,5,6,7,8,9,10 mixed with chicken (227bp) species, Lane 7 cattle, mixtures with 3 species, M100 :100 bp ladder, NC: control negative.

High adulteration value detected by Mosaad *et al.* (33) who found that oriental sausage samples contained meat from sheep, chicken, and equine animals at levels of 80%, 50%, and 10% respectively. Additionally, the analysis found seventy percent of beef luncheon samples were discovered to be adulterated with poultry, while ten percent contained equine species.

Despite the unprecedented rise in meat prices in Iraqi markets at the time of this study. The current results notice no positive samples have been detected for horse, dog and pork meat which represent the halal authentication, but it is possible if this excessive increase in prices continues due to the increase in demand and the decrease in supply of local meat.

These results complied with previous Al-Rashedi and Hateem, (35) Iraqi researchers who employing real-time PCR technique and mitochondrial

cytochrome b gene analysis to investigate pork contamination in commercial canned meat products in Iraq market tested, when twenty samples from the local market and the data were free of any pork detection in meat cans.

The current results were different with Hamouda *et al*. (36) who exam 48 products of raw kofta (minced), sausage, beef luncheon and beef burger samples and they notice the adulterated by dog meat were 33.3% and 66.7% in kofta (minced) and sausage. While the results were consistent and identical for equine and pig species, which were not detected in all samples. Conclusion

According to the findings derived from this study, it can be inferred that there was a significant level of bacterial contamination in all 100 samples of minced meat gathered from local markets in Baghdad city. Thus, it is advisable to incorporate the hazard analysis essential control system and Safe food practices when preparing and processing food. There was a significant level of the meat species fraudulent, therefore Multiple-PCR technique created during this study is highly sensitive, specific, and rapid, it is strongly recommended as an evaluation assay for detecting adulteration and violations of labeling requirements in meat products.

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