

Detection of *Escherichia coli* from Imported and Local Beef Meat in Mosul City

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Abstract

The aim of the present study is the detection of red beef meat (local and imported) contaminated by *E. coli*. The microbial load in local and imported beef meat was detected by counting the *E. coli* total number according to the universal standards. Sixty samples of local and imported beef meat from Indian origin were collected from city meat retailers and butchers that divided into 6 localities (quarters) including AL-Hadbaa, AL-Baladiat, AL-Faisaliala, AL-Majmoaa, AL-Jazair and AL-Wahdaa in Mosul city. Ten samples were collected from each locality (five local samples, five imported ones). All samples were subjected for morphological, biochemical (including API20E) and confirmed by molecular identification (PCR). Results were revealed that imported beef samples were more contaminated with *E. coli* bacteria than local samples since they ranged from $61 \times 10^2 - 22 \times 10^3$ CFU /g and 13×10^2 to 65×10^2 CFU / g respectively.

Keywords: PCR, *E. coli*, Bacterial count, Microbial contamination, Beef meat

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INTRODUCTION

World is growing up fast, therefore, the demands for finding other sources were observed; to insurance the protein requirements for next decades¹. The red meat consider as necessary food of human all over the world which be as one of the main sources of protein as well as lipid, vitamin, fats, mineral, and salts, also meat is one of the most valuable nutrients in human nutrition, the meat is the best media for growing deferent types of microorganisms causing fast spoilage of meat (food poisoning result from secretion of bacterial poison) leading to economic losses, the more important microorganisms which cause these loses are *Escherichia coli* (*E. coli*) and *Salmonella*²⁻⁴. *E. coli* in food is a pointer of bad sanitary situations⁵. Meat can become contaminated during slaughter or during processing. Infection can occur from eating contaminated undercooked meat, especially ground beef.

The *Escherichia coli* return to genus *Escherichia* which belong to family of *Enterobacteriaceae*, these bacteria are Gram-negative bacteria, fermenting the sugar and producing acid and gas without H₂S production. These bacteria have a big rate as a normal inhabitant in the gastrointestinal tract, also causes a disease to many species of animals, for example causes septicemia in lambs, horse, calf and dogs, so these bacteria consider as opportunistic bacteria⁶. Most important pathogens that play a role in food borne diseases are of animal origin, 2.2 million peoples each year were dead from food and water born diarrheal diseases (especially children) in developing countries⁷.

The aim of this study to evaluate the bacterial load on a local and imported beef meet and compare the total bacterial count of beef meat with the permissible limit of central organization and quality control.

MATERIALS AND METHODS

A-Sampling

In the current study, from June to November 2018, a total of 60 samples of beef meat collected from markets of Mosul city/ Iraq, included 30 samples from imported meat (Indian origin) and 30 samples from local meat were randomly collected. Samples were collected from local markets and booths, transported by sterilized

container to microbiological laboratory / College of Veterinary Medicine, University of Mosul, for examination

B-Microbiological analysis

Culturing and staining

All samples were cultured on the following media.

- Brain heart infusion broth, (LAB/UK)
- MacConkey agar (LAB/UK).
- Violet Red bile Glucose Agar {VRBGA (LAB/UK) (Media for enumeration of *Enterobacteriaceae* in food)}
- Eosin methylene blue (EMB, LAB/UK) for a metallic sheen phenomenon⁸.
- The bacterial colonies were stained by Gram's stain⁹.

Biochemical tests

Carried out according to a procedure^{10,11}. The biochemical tests included:

Indole production test, Methyl red, Voges proskauer, citrate Utilization, Urease test, Hydrogen sulphide production, oxidase test, and catalase test.

Detection of *E. coli* by API20 (Biomerix-France)

This method was used for the identification of the members of *Enterobacteriaceae*. The system has a capacity of 23 biochemical tests^{12,9}. In this method, 20 samples (10 samples from local and 10 samples from imported beef meat) were tested.

Bacterial counting

The total bacterial count was calculated using an aerobic plate count according to a procedure¹³, 25 g of sample was taken and added to 225 ml of buffer peptone water (BPW), mixed well for (3-5) min. Then decimal dilution was carried out up to the tenth dilution. 0.1 ml of each dilution was taken and placed on petri dish contained selective culture media (VRBGA, EMB) spreading well by glass spreader and incubated at 37°C for 24 hours { the medium of three Petri dishes from each diluted tube was performed (30-300 colonies)}, results of each dilution were read and fixed¹⁴. The results of bacterial counting accepted within the standard mean; when 3 of 5 samples were under the limited permissible account ($5 \times 10^2 - 5 \times 10^3$ CFU/g)¹⁵.

DNA extraction

Ten *E. coli* isolates were taken randomly from isolated bacteria, 5 isolates from local beef meat and 5 isolates from imported beef meat.

Extraction was done by using a specific kit (Jena Bioscience / Germany). isolates were cultured and incubated at overnight, then extraction was done according to the kit instructions as below:

Transfer 1ml of cultured cells into a 1.5 ml microtube, centrifuged at 15, 000 g for 1 min and discard the supernatant. Then re-suspend the pellet in 300 µl of Cell Lysis Solution. Added 1.5µl of RNase A Solution and mixed by inverting. Incubated at 37°C for 15-30 min and cooled on ice for 1 min. Then added 100µl of Protein Precipitation Solution and vortex vigorously for 20- 30sec. Again, centrifuged at 15, 000 g for 5min. After that transferred the supernatant to a clean 1.5 ml microtube containing 300 µl Isopropanol (99%), mix the sample by inverting gently for 1min.

Centrifuged at 15, 000 g for 1min (DNA should be visible as a small white pellet). The supernatant was discarded and tube drained briefly on clean absorbent paper. Then added 500µl Washing Buffer and inverted the tube several times to wash the DNA pellet. Final centrifugation was done at 15, 000g for 1min. After that, the ethanol was discarded carefully and let to air dry at room temperature for 10-15 min. Then added 50-100 µl of DNA Hydration Solution to the dried DNA pellet. Hydrated the DNA by incubating at 65°C for 60min. After that cooled to 4°C, and stored the sample at -80°C.

Polymerase chain reaction procedure (PCR)

Polymerase chain reaction for detection of *E. coli* was performed according to¹⁶. Specific primers were used in this study ECO223F (5'ATCAACCGAGATTCCCCAGT 3') and ECO223R (5'TCACTATCGGTGTCAGTCAGGAG3') (produced by IDT- USA). Amplification of DNA was performed in a total volume of 25µl containing: 12.5µl HS Prime Taq Premix (2X) (GeNetBio, Korea), 2µl of each forward and reverse primers, 6.5µl of PCR grade water and finally 2µl of DNA template. Positive and negative controls were also included in separate tubes. The mixture was subjected to amplification using thermocycler (QLS, UK) consisting of 1

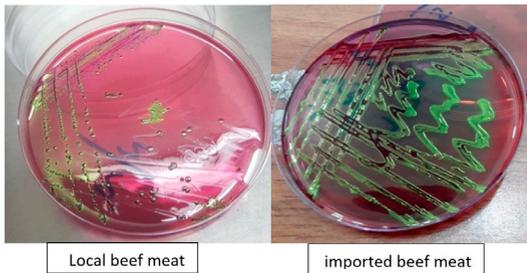


Fig. 1, 2. Growing of *E. coli* on EMB and producing Metallic sheen

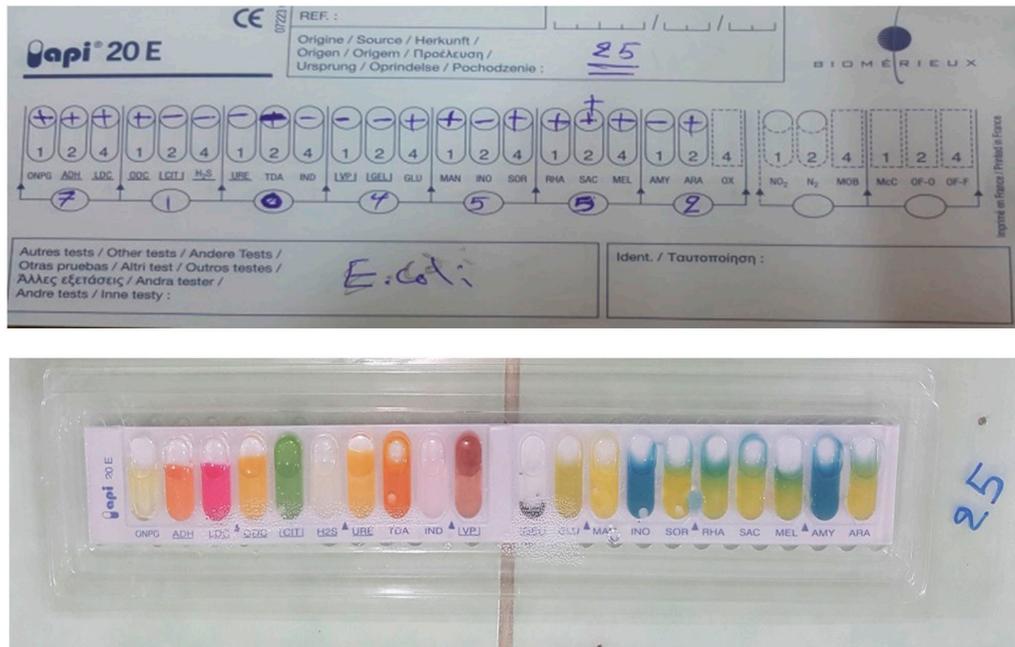


Fig. 3, 4. API20E for *E. coli* isolates

cycle of initial denaturation at 95°C for 10 min, then 30 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30sec and extension at 72°C for 1 min. The final extension was done at 72°C for 1min followed by cooling at 4°C forever. After amplification, the DNA products were analyzed by 1.5% agarose gel electrophoresis (Major Science, Taiwan) using 7 µl DNA ladder as a molecular weight marker (Gena Bioscience, Germany). The gel was visualized by UV transilluminator then

photographed by Gel documentation system (Biometra, Germany).

RESULTS

Bacterial isolation

Fifty-six samples were appeared positive for *E. coli* isolation (93.3%) according to selective media and results of biochemical tests and API20E (Fig. 1, 2), included 29 samples of local meat and 27 samples of imported beef meat.

Table 1. Bacterial count (cfu)/1g in local and imported beef meat

| Bacterial count in imported beef meat | | | | Bacterial count in local beef meat | | |
|---------------------------------------|---------|-----------------------|------------------|------------------------------------|-----------------------|------------------|
| Number of samples | origin | Bacterial count (cfu) | Places | Number of samples | Bacterial count (cfu) | Places |
| 1 | (India) | 120 x 10 ² | Jazair quarter | 1 | 81 ' 10 ² | Jazair quarter |
| 2 | (India) | 72 x 10 ³ | Jazair quarter | 2 | 78 ' 10 ² | Jazair quarter |
| 3 | (India) | 67 x 10 ² | Jazair quarter | 3 | No growth | Jazair quarter |
| 4 | (India) | 113 x 10 ² | Jazair quarter | 4 | 44 ' 10 ² | Jazair quarter |
| 5 | (India) | 80 x 10 ² | Jazair quarter | 5 | 122 ' 10 ² | Jazair quarter |
| Mean | | 22 x 10 ³ | | Mean | 65 X 10 ² | |
| 6 | (India) | No growth | Hadbaa quarter | 6 | 84'10 ² | Wahdaa quarter |
| 7 | (India) | 79 x 10 ² | Hadbaa quarter | 7 | 80 '10 ² | Wahdaa quarter |
| 8 | (India) | 47 x 10 ² | Hadbaa quarter | 8 | 90 ' 10 ¹ | Wahdaa quarter |
| 9 | (India) | 37 x 10 ³ | Hadbaa quarter | 9 | 62'10 ² | Wahdaa quarter |
| 10 | (India) | 104x 10 ² | Hadbaa quarter | 10 | 45' 10 ² | Wahdaa quarter |
| Mean | | 12 x 10 ³ | | Mean | 56 X 10 ² | |
| 11 | (India) | 109 x 10 ² | Wahdaa quarter | 11 | 13'10 ² | Faisalia quarter |
| 12 | (India) | 74 x 10 ² | Wahdaa quarter | 12 | 49 '10 ² | Faisalia quarter |
| 13 | (India) | 66 x 10 ² | Wahdaa quarter | 13 | 57 ' 10 ² | Faisalia quarter |
| 14 | (India) | 61 x 10 ² | Wahdaa quarter | 14 | 37 '10 ² | Faisalia quarter |
| 15 | (India) | 95 x10 ² | Wahdaa quarter | 15 | 104 ' 10 ² | Faisalia quarter |
| Mean | | 81 x 10 ² | | Mean | 52 X 10 ² | |
| 16 | (India) | 44 x 10 ² | Faisalia quarter | 16 | 12 ' 10 ² | Baladiat quarter |
| 17 | (India) | 62 x 10 ² | Faisalia quarter | 17 | 60 ' 10 ¹ | Baladiat quarter |
| 18 | (India) | 63 x 10 ² | Faisalia quarter | 18 | 78 ' 10 ² | Baladiat quarter |
| 19 | (India) | 80 x 10 ² | Faisalia quarter | 19 | 50 ' 10 ² | Baladiat quarter |
| 20 | (India) | 91 x 10 ² | Faisalia quarter | 20 | 89 '10 ² | Baladiat quarter |
| Mean | | 68 x 10 ² | | Mean | 47X 10 ² | |
| 21 | (India) | No growth | Baladiat quarter | 21 | 27 ' 10 ² | Hadbaa quarter |
| 22 | (India) | 93 x 10 ² | Baladiat quarter | 22 | 55 ' 10 ² | Hadbaa quarter |
| 23 | (India) | 87 x10 ² | Baladiat quarter | 23 | 73 ' 10 ¹ | Hadbaa quarter |
| 24 | (India) | 66 x 10 ² | Baladiat quarter | 24 | 17 ' 10 ² | Hadbaa quarter |
| 25 | (India) | 79 x 10 ² | Baladiat quarter | 25 | 38 ' 10 ² | Hadbaa quarter |
| Mean | | 65 x 10 ² | | Mean | 42 X 10 ² | |
| 26 | (India) | 103 x 10 ² | Majmoaa quarter | 26 | 50 '10 ¹ | Majmoaa quarter |
| 27 | (India) | 41 x 10 ² | Majmoaa quarter | 27 | 107 ' 10 ² | Majmoaa quarter |
| 28 | (India) | 86 x 10 ² | Majmoaa quarter | 28 | 88 ' 10 ² | Majmoaa quarter |
| 29 | (India) | 75 x 10 ² | Majmoaa quarter | 29 | 31 ' 10 ² | Majmoaa quarter |
| 30 | (India) | No growth | Majmoaa quarter | 30 | 140 ' 10 ¹ | Majmoaa quarter |
| Mean | | 61 x 10 ² | | Mean | 13 X 10 ² | |

Detection for *E. coli*, group of tests by (API20)

The isolates from local and imported beef meat were subject to farther more confirmative API reaction. Ten isolates from each of them gave the presumptive *E. coli* bacterial reaction, they are expressed in Fig. 3, 4.

Bacterial counting

The results of bacterial count showed that 4 localities out of 6 of local beef meat (30 samples) have an acceptable limits of *E. coli* number ($5 \times 10^2 - 5 \times 10^3$ CFU/g), while all imported beef meat (30 samples) (India) were out of ICSMF and EEC limits since they were $61 \times 10^2 - 22 \times 10^3$ CFU/g¹⁵. Fig. 5, 6, Table 1.

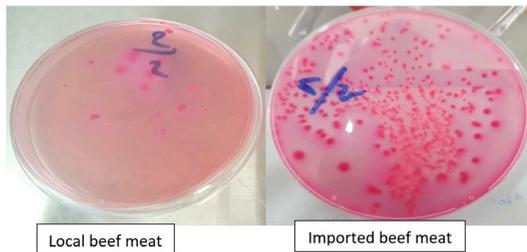


Fig. 5, 6. Bacterial count in local and imported beef meat

Polymerase chain reaction

The results of isolates confirmed by a polymerase chain reaction of ECO.223 gene of *E. coli* lane M.100 bp DNA ladder were used. Ten isolates were taken randomly (5 isolates from each local and imported beef meat), Lanes 1-5 are positive samples (2, 9, 13, 18, 25) from local beef meat. Lanes from 6-10 are positive samples (4, 10, 15, 23, 27) from imported beef meat. Lanes 11 is negative control and lanes 12 is positive control (Fig. 7).



Fig. 7. Polymerase chain reaction of ECO223 gene of *E. coli*. Lane M: 100 bp DNA ladder. Lane 1-10 are positive samples, lane 11 negative control and lane 12 positive control.

DISCUSSION

Meat is a perfect source of protein in the human diet and it is greatly vulnerable to microbial contaminations, which can cause food poisoning and foodborne infections in human¹⁷.

Red meat production is an essential and important livestock production since the livestock production contribute about 22% agricultural production (except fish) in Iraq from 2009 to 2013⁴.

The founding of bacteria in meat has been reported universally, most of the pathogens which play a major role in foodborne diseases have a zoonotic origin^{1,17}. Globally, a number of studies have been conducted in this field, show special importance to bacterial contamination to red meat. The results of the current study were revealed that the imported meat was highly contaminated and unsatisfied for human consumption ($61 \times 10^2 - 22 \times 10^3$ CFU/g), while the local samples appeared more acceptable when compared with universal standards (13×10^2 to 65×10^2 CFU/ g). These results confirm that local meat is more fitness for consumption. The results of a local study², were closed to the present study in term of fact that imported red meat is more polluted than local red meat and attributed to the contamination of *E. coli*, which may be due to a poor health condition in slaughterhouses and carcasses or during processing. Also, the present study was agreed with the results of the study that carried out by³, which revealed that imported meat was more contaminated than local meat in term of coliform bacteria and deferred from the study carried by¹⁸ in case of elevated total number of *E. coli* bacteria.

The current study was disagreement with the study done by¹⁹, which say that no differences in microbial load between the local and imported meat. Meat preservation is necessary during transporting for varied distances to reduce spoiling of texture, color, and nutritional value after the development and rapid growth of supermarkets¹.

Also, the present findings have some agreement in general with the study done by¹⁸ in case of that the highest number of coliform bacteria. So, it was stressed that the local beef meat is more preferable to be on our tables than the imported beef meat. To control the foodborne illnesses and to keep the microbial load of

raw meat in check, the food safety requirements should be followed hardly Hazard analysis critical control point^{17,19}.

CONCLUSION

Present study indicated that microbial load of *E. coli* contamination was higher in imported meat (India) than local meat and these may be due to transportation, long period of storage with intermittent electrical cut (bad sanitary condition) and don't follow the health condition.

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FUNDING

None.

DATA AVAILABILITY

All datasets created or investigated during this study are involved in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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