

## A Microbial Study of Minced Meat and Raw Kebabs in Some Restaurants and Its Relationship to the Cultural and Health Level of Its workers

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### Abstract

This study is carried out to investigate and evaluate the level of cultural and health awareness of workers in some restaurants in the city of Karbala. And the extent of their application of health conditions in preparing kebabs for consumers and its relationship to the microbial content. It was found through the questionnaire forms and the personal interviews that the health conditions weren't applied, due to poor health awareness, and lack of knowledge of the factors of food contamination with microbes. Therefore, microbial contamination in the kebab was investigated during its manufacturing stages. The study included randomly selecting 10 restaurants in a center of the city centre and surrounding areas, the number of samples studied were 20 samples of minced meat and 20 samples of raw kebabs for the period from 1/7-28/7/2021 (total samples 160), the microbial tests included, total plate count (TPC) and total coliform bacteria (TC) and *Staphylococcus aureus* (SA), as well as *Salmonella* test. The result of this study showed that TPC, TC, SA, in minced meat is  $10.1 - 13.7 \times 10^7$  CFU / g,  $11.9 - 15.1 \times 10^5$  CFU / g,  $5.3 - 9.1 \times 10^5$  CFU / g, respectively. *Salmonella* test was negative in all samples. And in raw kebab were  $11.2 - 15.2 \times 10^7$  CFU / g,  $12.6 - 16.9 \times 10^5$  CFU / g,  $6.1 - 10.2 \times 10^5$  CFU / g, respectively. *Salmonella* test was positive in 16 samples in minced meat from 80 samples, during the study period (the Percentage of contamination was 20 %). And all of kebab's meat were negative identified or did not contain *E. coli*, *Staphylococcus aureus*, and *Salmonella*, after the grilling process. When comparing these results with the microbial limits of the Iraqi standard (2006), it was found that more than 70% of the samples contaminated exceeded the maximum limit. We also identified, through this study, the weakness of the cultural and health level of some restaurant workers with regard to preparing kebabs in the correct way.

**Keywords:** Microbiology, Raw Kebabs, Contamination, Minced Meat, Salmonella.

### دراسة المحتوى الميكروبي للحوم المفرومة والkebabs النيئ في بعض المطاعم

#### وعلاقته بالمستوى الثقافي والصحي للعاملين فيها

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### الخلاصة

اجريت هذه الدراسة لمعرفة وتقييم مستوى الوعي الثقافي والصحي للعاملين في بعض المطاعم في مدينة كربلاء ومدى تطبيقهم للشروط الصحية في تحضير kebabs للمستهلكين وعلاقتها بالمحتوى الميكروبي. وتبين من خلال استمارات الاستبيان والمقابلات الشخصية عدم تطبيق الشروط الصحية، وضعف الوعي الصحي بمعرفة عوامل تلوث الغذاء بالميكروبات. تم دراسة التلوث الميكروبي في kebabs خلال مراحل تصنيعه حيث شملت الدراسة 10 مطاعم اختيرت عشوائياً في مركز المدينة والمناطق المحيطة بها، وكان عدد العينات المدروسة 20 عينة لحم مفروم و20 عينة كebab نيئ للفترة من 2023/7/28-7/1 (مجموع العينات الإجمالي 160)، شملت الاختبارات الميكروبية، العد الكلي للصفائح (TPC) والبكتيريا القولونية الكلية (TC) والمكورات العنقودية الذهبية (SA)، وكذلك اختبار السالمونيلا. أظهرت نتائج هذه الدراسة أن TPC, TC, SA في اللحم المفروم هي  $10.1 - 13.7 \times 10^7$  CFU / g,  $11.9 - 15.1 \times 10^5$  CFU / g,  $5.3 - 9.1 \times 10^5$  CFU / g, وعلى التوالي. وكان اختبار السالمونيلا سلبياً في جميع العينات. وفي kebabs الخام كانت  $11.2 - 15.2 \times 10^7$  CFU / g,  $12.6 - 16.9 \times 10^5$  CFU / g,  $6.1 - 10.2 \times 10^5$  CFU / g, وعلى التوالي. وجاء اختبار السالمونيلا إيجابياً في 16 عينة في اللحم المفروم من أصل 80 عينة خلال فترة الدراسة (أي أن نسبة التلوث كانت 20%). وقد تم تشخيص جميع لحوم kebabs بأنها سلبية أو لا تحتوي على الإشريكية القولونية والمكورات العنقودية الذهبية والسالمونيلا بعد عملية الشوي. وعند مقارنة هذه النتائج مع الحدود الميكروبية للمعيار العراقي (2006) وجد أن أكثر من 70 نسبة العينات الملوثة تجاوزت الحد الأقصى. كما تعرفنا من خلال هذه الدراسة على ضعف المستوى الثقافي والصحي لدى بعض العاملين في المطاعم فيما يتعلق بإعداد kebabs بالطريقة الصحيحة التي لا تؤثر على صحة المواطن.

## **1. Introduction**

Due to the importance of the city of Karbala in religious tourism, it has become one of the most famous cities in Iraq that receives visitors. Consequently, there was a significant increase in the number of restaurants, and unfortunately, some restaurants do not pay attention to food safety during its preparation, which leads to an increase in the chances of food contamination and as a result leads to the possibility of transmitting diseases to restaurant guests. Since the provision of healthy food is the responsibility of the workers in restaurants, so educating and sensitizing them is the main goal to secure adequate food protection from pollution [1].

Despite the presence of several government institutions responsible for health control in restaurants, but this cannot solve the problem of food contamination in the absence of health and nutritional awareness of restaurant workers. Therefore, particular specifications and standards must be set for health conditions in restaurants, and the need to ensure the granting of health licenses to them, along with the medical examination of their workers to ensure their safety from diseases. Meat and its products are great importance in human nutrition. It is one of the main sources of protein in addition to fats and some other mineral elements. The red meat in Iraq occupies the first place when compared with the total of other meat [1] [2].

Meat and its products are suitable mediums for the growth and reproduction of microorganisms, due to the nutrients they contain and sufficient moisture [2] therefore they can be exposed to microbial contamination from slaughtering to preparation and manufacturing. The degree of meat contamination is determined by knowing the numbers and types of microorganisms growing in it. Meat is not free of microorganisms, but its numbers may increase if certain conditions are available, such as temperature, humidity, and oxygen [3] and [4]. The number of microorganisms in meat also increases as a result of contamination during the process of slaughter, cutting, preparation, and manufacturing, moreover, The poor storage conditions, repeated thawing process, mincing machines and tools in restaurants, and the hands of workers are all considered factors that increase the number of microorganisms in meat [5]. Minced meat is more suitable for the growth of these microorganisms due to the liberation of juices in the tissues and the increase in the surface area, and thus the microorganisms are in direct contact with the components of the meat, causing accelerated damage.

The extent of contamination of meat can be known by estimating the numbers of microorganisms and comparing them with the limits allowed by local or international Standard Specifications. Among these tests is the determination of the total number of bacteria carried out by the Total Plate Count (TPC) method, which is considered one of the microbiological tests approved in evaluating the health level of various foodstuffs [6].

As for the estimation of total coliform bacteria (TC), this group are considered to be of great importance in terms of health, and the presence of coliform bacteria in foods is considered undesirable, and it is evidence of faecal contamination of food, and evidence of the possibility of the presence of pathogenic bacteria in those foods, in addition to that Coli bacteria have a large role in food spoilage. [7] and [8]. The estimation of staphylococcus aureus is also important because the presence of this bacterium in food causes staphylococcus poisoning, and this is one of the most common types of food poisoning. It is necessary to conduct detection of salmonella bacteria because all types of these bacteria are pathological, and the number of poisoning infections with such bacteria in many countries reaches 7-10 million infections annually [9], [10], [11] and [12] from isolates of Salmonella bacteria with different percentages from chicken and beef samples, especially minced meat.

## 2. Material and Methods

Samples: 20 samples of minced meat and 20 samples of raw kebab were collected for the period from 1\7-28\7\2021 (total samples 160) and placed in polyethylene bags in the freezer at -18°C until bacteriological tests were performed.

Bacteriological tests: Samples were analysed by microbial methods based on the method of [13] in bacteriological tests, where 25 g of minced meat was weighed and mixed with 225 ml of Peptone Water nutrient broth medium in an electric mixer for two minutes at a speed of 230 revolutions/min, and this is the first dilution 10<sup>-1</sup>, then the rest of the dilutions were completed using Peptone Water (PW) medium. 9 ml of medium was placed in each tube, and 1 ml of the first dilution was added to it to become 10<sup>-2</sup> dilution, and so on until dilution 10<sup>-4</sup>. The same was done for raw kebabs. These dilutions were used to estimate the bacteriological quality, which included:

Test the Total Plate Count (TPC) A Plate Count Agar medium, where 1 ml of the appropriate dilution was transferred to a petri dish with a sterile pipette then pour the medium after cooling it to 45 °C and stir the dishes quietly for homogeneity and distribution well, It was allowed to harden, then the plates were inverted and incubated at 37 °C for 24 hours, after which the number of bacteria growing in the plates was counted [14].

Test the total coliform bacteria (TC): The MacConkey agar medium was used to detect coliform bacteria. The medium was poured into the dishes and allowed to solidify. Then 1 ml of the appropriate dilution was placed on the medium and spread well on the surface, then another layer of the medium was poured over it in order to provide anaerobic conditions and the dishes were left to solidify, then turned and incubated at 37°C for 24 hours, The colonies growing on the medium were counted to estimate the total number of coliform bacteria.

Staphylococcus aureus: The diffusion method was used, where 0.1 ml of the appropriate sample dilution was taken and placed on the surface of the solidified Baird parker Agar in Petri dishes and spread on the surface of the hardener , The dishes were incubated at 37 °C for 48 hours, and the total number of them was estimated.

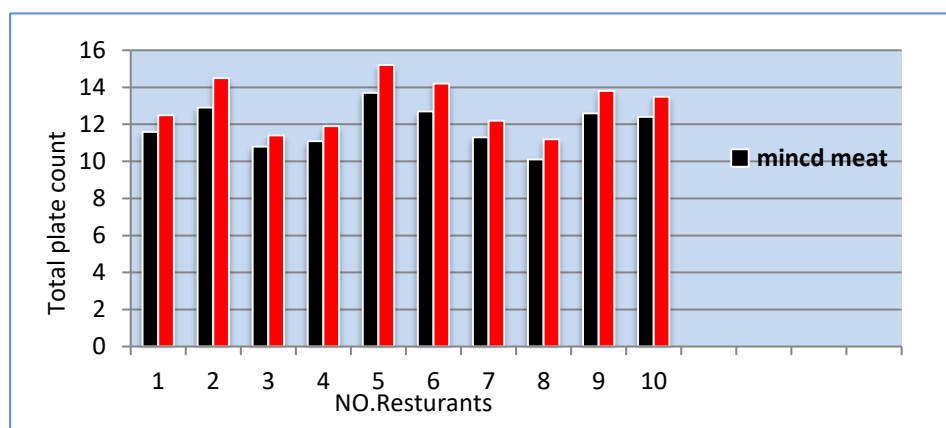
Test Salmonella Bacteria: Salmonella spp test was carried out using the pour plate method with Salmonella shigella agar (SSA) media suspension then poured into selective salmonella shigella agar (SSA) media. The incubation of the culture with the position of the lid of the petri dish remained above, and then the cup was reversed. After being incubated at 37°C for 24 h, a red dot black colony is found in the centre [15].

## 3. Results and Discussion

### 3.1 Total Bacteria

Figure 1 shows the total bacterial number (TPC) in minced meat and raw kebab ranges from 10.1 -13.7 x 10<sup>7</sup> CFU/g. 11.2- 15.2 x 10<sup>7</sup> CFU / g respectively. It appeared that more than 65% of the samples had (TPC) content above the limits of (Iraqi Standard system No. 2006/2270), which determined the total number of aerobic bacteria in meat from (10<sup>6</sup>-10<sup>7</sup> CFU/gm), and it was more than the limits of the international standard that limits of (TPC ) number (10<sup>4</sup>-10<sup>6</sup> CFU/gm). The results of the this study are similar to the results of [1], in their study on local and imported red meat, and the results of [14] in their study on pork and [15], in their study on minced red meat. Authors in [6] in their study on the microbial content of beef. While these results were less than the results of the study of [16] when it was tested on beef used in making

kebabs and [5], on raw and minced meat in Canada, and [2], in his study on beef, and [17], in their study on red meat products in the Egyptian markets and [18] which found that the average (TPC) of minced beef ( $8.1 \times 10^8$  CFU / g). It was also found through the results of the above study that the percentage of contamination in raw kebab dough is more than in minced meat, and the reason may be attributed to the role played by the worker in preparing kebabs. It was also shown through the personal interactions of restaurant workers and the questionnaire that includes 20 questions, the weakness of health control by the concerned authorities, and the weakness of interest in following health conditions by workers in most restaurants in preparing, manufacturing and serving kebabs. With not following the correct procedures in cleaning utensils and tools for preparing kebabs, in addition to not conducting a periodic medical examination for workers.)



**Figure -1** Total Bacteria in minced meat and raw kebab

### 3.2 Total Coliform (TC)

It was shown through Figure 2 that all the studied samples were also contaminated with coliform bacteria in varying proportions, and it appeared that the percentage of contamination in raw kebabs is more than in minced meat. Where the number of coliform bacteria (T.C) in minced meat and raw kebab ranged from ( $11.9 - 15.1 \times 10^5$  CFU/gm), ( $12.6 - 16.9 \times 10^5$  CFU / g), respectively. The (TC) in all samples was higher than the microbial limits specified by the international standard that determined the number of (TC) from ( $10^1$ - $10^3$  CFU/gm of meat) [12], although it was in agreement with the results of [19], when they studied the microbial content of ground meat. And some of the results of [6] when studying the bacteria numbers in local and imported red meat and the results of both [3] and [8], and are close to the results of [3]. While these results were higher than the results of [20], and the results of [7], in their study on chicken thighs and [15], in their study on minced meat, and [4], and [12], who found *E. coli* in 50% of the minced meat samples and 30% of the kofta and beef burger samples. This contamination has dangerous indicators on human health. The cause of the contamination may be attributed to coliform bacteria, especially *E.coli*, due to the lack of personal hygiene for restaurant workers, in addition to poor handling and storage, frequent thawing and freezing due to frequent power outages, with weak health control. [14], pointed out that poor handling, slaughter, skinning and storage does not prevent fecal contamination.

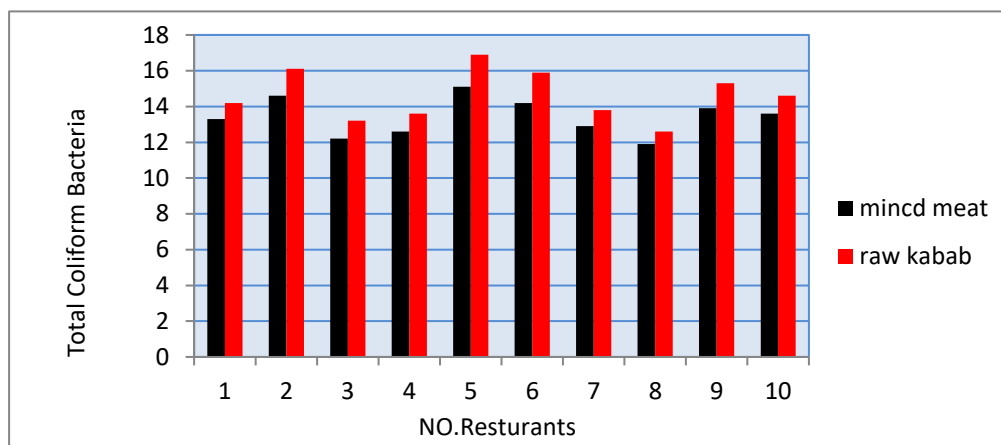


Figure -2 Total Coliform (TC) in minced meat and raw kebab

### 3.3 Total number of *Staplaylococcus aureus*

Figure 3 show that all studied samples were contaminated with *S.aureus* bacteria, and the contamination in raw kebabs was more than in minced meat, and their number in minced meat and in raw kebabs ranged between  $(5.3 - 9.1 \times 10^5 \text{X CFU / g})$  ,  $(6.1 - 10.2 \times 10^5 \text{ CFU / g})$  respectively. The results of this study agreed with the results of [19], [17] and [1], while it was higher than the limits allowed by (Iraqi Standard No. 2006/2270), which specified The number  $(1 \times 10^3 \text{ CFU / g})$ , as it was higher than the permissible limits in the international standard that determined the total number of *Staphylococcus aureus* bacteria  $(1 \times 10^2 - 1 \times 10^4 \text{ CFU / g meat})$  . And it is higher than the results of [7] in their study on chicken thigh for some companies. While it was less than the results of [4], in their study on the microbial content of sausages in the Egyptian markets. The cause of contamination with *s.aureus* may be caused by the workers in the restaurant through the hands, sneezing and coughing, during the preparation of kebabs. And it is possible for contamination to occur by tools and equipment used in cutting and mincing meat, as the contamination is transferred from one substance to another [21].

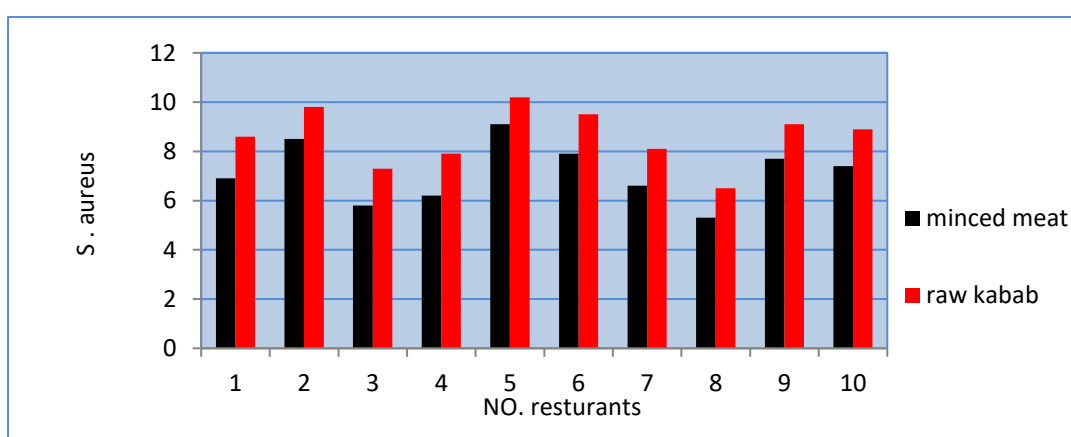


Figure 3- Total number of *Staplaylococcus aureus* in minced meat and raw kebab

The results of the study, as in Table 1, Salmonella test was positive in 15 samples in minced meat from 80 samples, (That is the Percentage of contamination was 19 %.), and in 19 samples in raw kebab from 80 samples, during the study period (That is the Percentage of

contamination was 23.75 %.), which indicates the exposure of kebab meat to infected people or carriers of this bacteria. This result is close to results was found by [20] and [21], but were higher than the result of research conducted by [8] and [9]. While these results were less than the results of [19], where he was indicated in his study that the percentage of contamination in raw kebab was 50% , and less than the results of [18] who confirmed that most poultry meat in developed countries is infected with *Salmonella*, as well as [22] who were isolated salmonella bacteria from 20% of the minced meat samples and 10% of the beef burger samples, and [16], found 1 out of every 4 samples of meat kebab is contaminated with *salmonella* the presence of this bacteria in minced meat and raw kebab may be attributed to contamination from the hands of workers Also and kebab preparation machines and equipment (Cross Contamination). Table 1 shows and all samples of grilled kebab's meat were negative identified or did not contain *E. coli*, *Staphylococcus aureus*, and *Salmonella*.The grilling process may be the reason for killing microbes, thus the grilled kebab is free of contamination.

**Table 1-** Samples numbers of *Salmonella* sp in Minced meat, raw and grilled kebab

Samples	Number of samples	Positive samples	Negative samples	Percentage of contamination
Minced meat	80	65	15	18.75 %
Raw kebab	80	61	19	23.75 %
grilled kebab	80	80	0	0 %

#### 4. Conclusions

The studied samples of minced meat and raw kebab were not in conformity with the microbiological standard specifications, and these results can be considered as a dangerous indicator because of the non-compliance with the health conditions in most of these restaurants. Ensure that there is a technical, cultural and health deficiency among restaurant workers in the preparation of kebabs, and the Basics of food preservation to protect them from contamination. Some restaurant workers do not have information about pathogenic microbes and their risks to the health of the citizen. Personal hygiene and the cleanliness of equipment and machines in preparing kebabs are not at the required level. A defect was observed in the continuous health control and the lack of periodic medical test for restaurant workers.

#### 5. Recommendations

Participation restaurant workers in training courses to increase their knowledge of the basics of food protection from contamination. Increasing the supervisory staff to ensure raising the level of health control, and we also recommend increasing the number of inspection visits on a regular basis.

Necessity of conducting an annual medical test for restaurant workers to ensure their safety from diseases. Using the media, newspapers and magazines and distributing them to restaurant owners to contribute to health awareness for food preparation. It is necessary for all restaurant workers to obtain a health certificate proving that they are safe from diseases.

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