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Isolation and identification of *Escherichia coli* and *K pneumonia* and Salmonella bacteria isolated from frozen meat in Basra Governorate

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Abstract

Meat is a primary source of proteins, vitamins, and minerals; however, it is prone to contamination with pathogenic bacteria during production and storage processes. Among the most common bacteria in frozen meat are *Escherichia coli*, *Klebsiella pneumoniae*, and Salmonella spp., which are significant contributors to foodborne illnesses and infectious diseases in humans**.**

A total of 60 random samples, including imported beef, chicken, and fish (20 samples each), were collected from different regions in Basrah. Bacterial identification was carried out using differential and selective media in combination with the Vitek®2 system. The results showed that 17 samples (28.3%) tested positive for *E. coli*, while 6 samples (10%) were contaminated with *K. pneumoniae*. No Salmonella spp. were detected in any of the samples. Additionally, 24 samples (40%) contained undiagnosed bacteria, and 13 samples (21.7%) exhibited no bacterial growth

Keywords: Meat contamination, *Escherichia coli*, Klebsiella *pneumonia*e, foodborne pathogens, Basrah, contained undiagnosed bacteria

1. Introduction

Meat is used all over the world as a necessary food for humans. It is the most important source of protein, in addition to the fats, salts, vitamins and minerals it provides, as it has great importance for health due to the polyunsaturated fatty acids it contains [1]. Red and white meat can become contaminated at any stage of the production process,from skinning,plucking, cutting, washing and even refrigerated or frozen storage [2]. This raw meat is an ideal breeding ground for a wide range of microorganism making it susceptible not only to spoilage but also to contamination with foodborne pathogen such as *E.coli*, *Staphylococcus aureus*, *Listeria*, *Campylobacter* or *Salmonella* which cause illness and death worldwide, especially in developing countries [3, 4].

Most frozen meat is stored at temperatures between (-18 - -30 °C), and the duration of meat storage depends on the type of animal, the freezing temperature, its stability, and the quality of the encapsulating material. The effectiveness of bacteria stops at a temperature of (-10 °C), while all chemical activities stop at a temperature of (-80 °C). It is difficult to provide this temperature because it is not commercially economical. Moreover, the process of defrosting meat gives a greater opportunity for bacteria to multiply and grow [5].

Vegetative cells of microorganisms die at the appropriate freezing temperature, as they cannot reproduce. As storage continues, the numbers of microorganisms decrease and some species die, while the numbers of some types of bacteria increase when stored at a temperature of (-4) °C to (-7) °C 70.% of *E.coli* cells survive at these temperatures [6].

Although the Ministry of Commerce in Iraq imposes strict laws on the import of different types of meat, such as Legislation No. 56 of 1983, some merchants import meat from unknown origins or the storage conditions are incorrect, causing the meat to be contaminated with various types of bacteria.

The aim of this study is to detect the presence of *E.coli*, *K. pneumonia* and Salmonella bacteria in different types of local and imported frozen meat in different areas in Basra Governorate.

Method

The study included collecting (60) samples of imported frozen meat (beef meat, chicken meat, and fish meat, 20 samples each) from stores selling frozen meat in different areas of Basra Governorate (Qibla, Karma, Safwan, Medina, Old Basra) for a period of one month during the period. From 2/24 to 3/22 of 2024. The sample size ranged from 50-100 grams, to obtain good results, the samples were placed in an insulated ice box under sterile conditions without delay.

The samples were transferred to test tubes, to which 15 ml of buffered peptone water was added, which had been previously sterilized in an autoclave at a temperature of 121°C and a pressure of 1.5 atmospheres. The sample was shaken for five minutes, and (1-2) ml of buffered peptone water was taken from each sample and added to Pre-prepared tubes containing 10 ml of Nutrient broth and Selenite Cystine Broth. The tubes were incubated at 37°C for 24 hours. After the end of the incubation period, the samples on Nutrient broth medium were transferred to McConkey Agar, and the samples on Selenite Cystine Broth medium were transferred to Salmonella-Shigella Agar and incubated at a temperature of 37° for 24 hrs [7, 8].

The lactose-fermenting pink colonies were transferred to EMB medium and incubated under the same conditions. The bacteria were identified using a device Vitek® [2].

Result

17 (28.3%) samples of *E.coli* bacteria were isolated, distributed as follows: 6 (35.3%) samples from beef meat, 5 (29.4%) samples from chicken meat, and 6 (35.3%) samples from fish meat out of the total number of samples. 60 samples, while 6 (10%) of *K. pneumoniae* bacteria were isolated and were distributed as follows: 4 (66.7) samples from fish meat and 2 (33.3%) samples from chicken meat, while this bacteria did not appear in the meat samples.

24 (40%) of the samples showed undiagnosed bacteria, as follows: 9 (37.5%) samples of chicken meat, 7 (29.6%) samples of fish meat, and 8 (33.3%) samples of beef meat, while 13 (21.3%) did not appear. The samples showed no growth of bacteria and were as follows: 3 (23.1%) red meat samples, 6 (46.2%) chicken meat samples, and 4 (30.7%) fish meat samples, while the results did not show any growth of salmonella bacteria.(Table no.1, Fig (1))

Table 1: The numbers of *E.coli* bacteria, *K. pneumonia*, other bacteria, and samples that did not show any growth, along with their percentages.

Bacteria / Sample type		***E.coli 17* (28.3)**		**K. *pneumonia 6)* (10%**		**Undiagnosed bacteria 24(40%)**		**No grouth 13 (21.3%)**		**Salmonella. Spp.**
	no	%	No	%	no	%	no	%	no	%
meat	6	35.3	0	0	8	33.3	3	23.1	0	0
chicken	5	29.4	2	33.3	9	37.5	6	46.2	0	0
Fish	6	35.3	4	66.7	7	29.2	4	30.7	0	0
Total	17	%100	6	%100	24	%100	13	%100		%0

It is clear from the table and chart above that the most contaminated types of meat were undiagnosed bacteria, with the highest infection rates recorded, followed by contamination with *E.coli* bacteria, then *K. pneumonia*. The results also showed that the meat most contaminated with different types of bacteria is fish meat, followed by chicken meat, then beef.

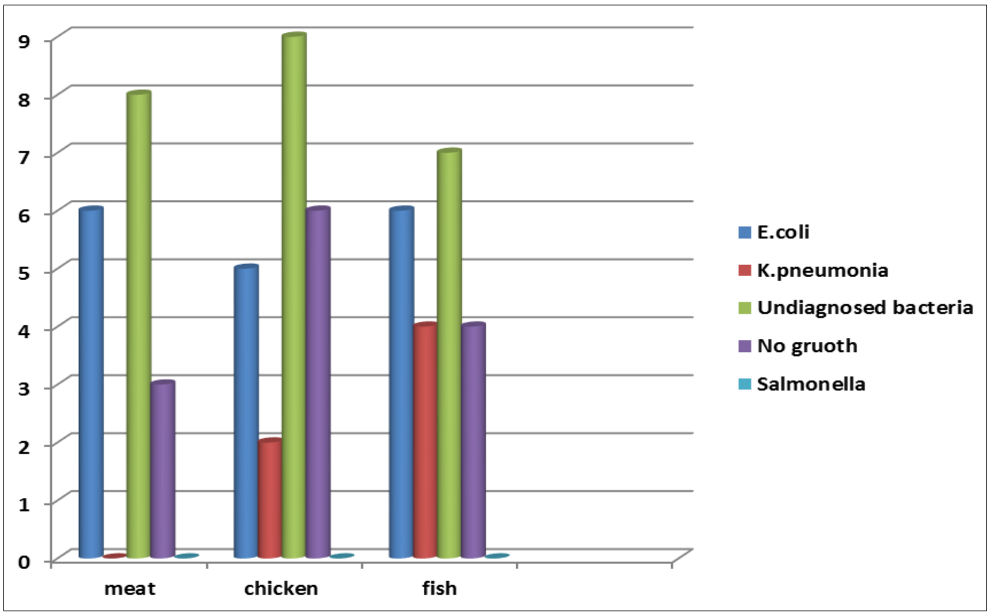

Fig 1: The type of meat and the bacteria that were isolated from it (*E.coli, K. pneumonia, other bacteria, no bacteria*)

In table (2) and Figure(2) It was found that the most contaminated samples were in the Old Basra, where the number of samples reached 14 cases, followed by the Karma Ali region, where the number of samples was 13 samples, then Al- Qibla and Safwan regions, with 12 contaminated samples, and the least was Al- Medina region.

Table 2: Shows the location of sample collection, the number of (*E.coli*, K. *pneumonia*, other bacteria, no bacteria) and their percentages.

Bacteria Sample collection locations	*E.coli*		K.*pneumonia*		Undiagnosed bacteria		No grouth		
	No.	%	No.	%	No.	%	No.	%	Total
Al-Qibla	3	17.6	0	0	8	33.3	1	7.7	12
Old Basra	4	23.5	2	33.3	4	16.6	4	30.8	14
Al-Medina	2	11.8	1	16.7	3	12.5	3	23	9
Safwan	3	17.6	2	33.3	4	16.7	3	23	12
Karma Ali	5	29.4	1	16.7	5	20.8	2	15.4	13
Total	17	100%	6	100%	24	100%	13	100%	60

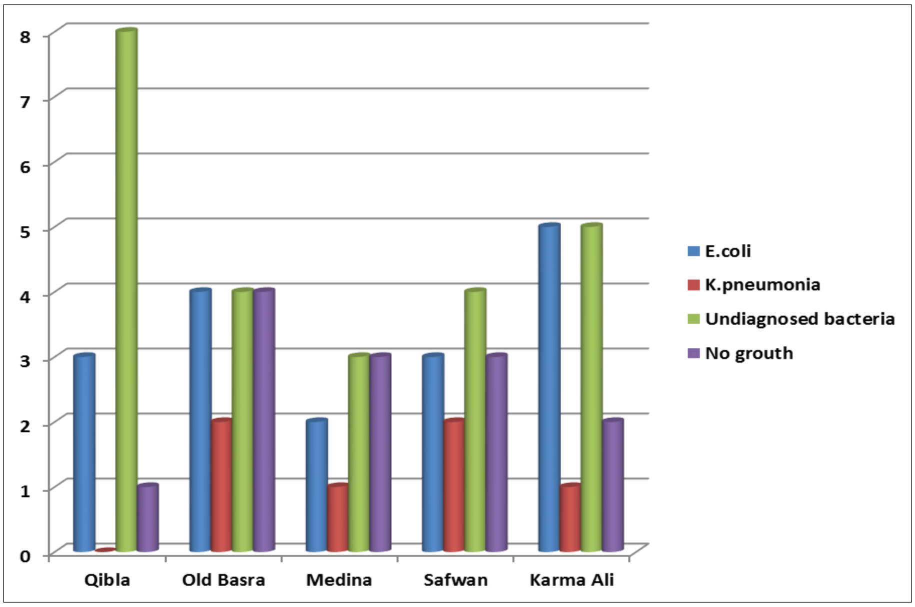

Fig 2: Shows the location of sample collection and the number of (*E.coli, K. pneumonia*, other bacteria and the no grouth)

Discussion

Our study aimed to isolate and diagnose *E.coli*, k. *pneumonia* and Salmonella spp. bacteria that may be present in various frozen meats isolated from different areas in Basra Governorate, because meat is considered one of the most important causes of food poisoning, as well as a source of gastroenteritis in humans [9].

Salmonellosis is a serious ongoing health problem. It has a major economic impact on the livestock sector in all countries of the world [10].

The traditional methods used to detect *E.coli and k. pneumonia* depend on enriching samples in buffer pepton water, which is preferable to use with this type of sample because the pH in it is neutral, and this is an important contributing factor in activating many types of bacteria, the first of which is *E.coli*. Therefore, the pH does not decrease in the peptone buffer water, so it gives the damaged cells an opportunity to regain their vitality before exposure to the culture media [11]. This is followed by dispersing the bacteria on MaCconky medium, then cultivating them on selective media on eosin methylene blue (EMB) agar, then subjecting them to chemical tests, thus confirming the correct diagnosis of the bacteria. The diagnosis is also confirmed using PCR technology, which we could not use due to its lack of availability and high price [12].

Vitek 2 is one of the best devices to identify all types of pathogenic bacteria and yeasts very accurately and within a short period, learn about their classification, and test their degree of sensitivity to antibiotics [13, 14].

The presence of *E.coli* in frozen meat may pose a risk to workers and consumers, and this is consistent with (Abuelhassan, *et al.* 2014) who explained that the presence of pathogenic *E.coli* bacteria is a potential risk to human health due to the ability of *E.coli* to survive in frozen beef [15].

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