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UDC579.672 **Research article**

Analysis of microbial contamination in different types of meat in the Kostanay region

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Abstract

Background and Aim. Meat is a favourable environment for the growth and viability of pathogenic microorganisms. Bacteria in meat cause spoilage and increase the risk of foodborne toxic infections of various origins during consumption. The importance of monitoring and analysing microbial contamination is increasing due to increased requirements for food safety and public health protection. The aim of the study was to analyse the overall level of microbial contamination of different types of meat sampled from retail outlets in the Kostanay region.

Materials and Methods. A total of 30 meat samples, including pork, beef, and horse meat, were collected for analysis. To assess microbial contamination of the meat, the number of mesophilic aerobic and facultatively anaerobic microorganisms (QMAFAnM) and their species composition were determined.

Results. It was found that 96% of the meat sold at the retail level had elevated QMAFAnM, with coliforms isolated in 30% of the samples. *Salmonella spp.* and *Listeria monocytogenes* were not detected in the meat tested. Pork meat had the highest microbial contamination compared to beef and horse meat samples.

Conclusion. Exceeding the permitted levels of QMAFAnM and the presence of coliform bacteria indicate potential health risks for consumers and more effective measures are required to ensure food safety.

Keywords: coliforms; food safety; meat; microbial contamination; QMAFAnM.

Introduction

Food safety and consumer protection are fundamental priorities in government policies worldwide. Ensuring the safety of raw materials and food products is essential for maintaining public health. Food quality directly impacts population health, helping to reduce the risk of infectious and chronic diseases. This underscores the need for strict control over the production, storage, and distribution of food products.

Meat is an important component of a healthy diet, rich in high-quality protein essential for the normal functioning of all body systems [1]. The proteins in meat provide all the essential amino acids necessary for the growth and repair of tissues. Additionally, meat contains B-group vitamins, which play crucial roles in energy metabolism and various metabolic processes, helping maintain a healthy nervous system and cognitive abilities [2]. Meat is also abundant in micronutrients like magnesium, selenium, and zinc that support the immune system and are involved in producing hormones and enzymes [3].

Meat also supplies bioavailable forms of iron and phosphorus, which are essential for oxygen transport and bone mineralization, respectively [4]. The unique nutrient composition of meat makes it a key dietary component for sustaining cellular functions and biochemical processes critical to physiological health.

However, it should be noted that meat and meat products can potentially source several infectious diseases. In particular, meat derived from animals afflicted with illness has the capacity to result in human infection with zooanthroponotic diseases [5, 6].

The contamination of meat with microbial organisms represents a significant challenge to consumer health and the quality of food products in the livestock and food industries. Pathogenic bacteria, including *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus*, may be present in poultry, pork, mutton and beef. Shiga-toxin-producing E. coli is frequently associated with beef and is responsible for a range of severe toxic infections, including haemorrhagic colitis and haemolytic uremic syndrome [7]. *L. monocytogenes* is a facultative anaerobic Gram-positive bacterium that is found in fresh meat and meat products. However, human listeriosis is mainly associated with ready-to-eat meat products with a long shelf life in the refrigerator [8]. A prevalence of 35% was observed for S. aureus in retail meat samples collected in 39 cities in China between 2011 and 2016 [7].

Furthermore, the contravention of storage conditions gives rise to the deterioration of foodstuffs and an augmented probability of contracting food poisoning upon consumption [7, 9]. Statistical evidence indicates that meat spoilage is responsible for approximately 400,000 deaths and over 600 million disease cases annually. The World Health Organization (WHO) has reported that a significant proportion of the global population has experienced food-related illness at some point in their lives, with over 30 diseases linked to poor food quality [10]. The relevance of this issue is also confirmed by data from Kazakhstan. In 2020, 37 cases of acute intestinal infections per 100,000 people were recorded, while in 2023, this figure increased to 55.37 cases per 100,000 people. The rise in morbidity highlights the need to tighten control over the quality of food products and improve sanitary and hygienic conditions at all stages of meat production and distribution [11].

The aim of our research is to analyse the overall level of microbial contamination of meat of different types of animals sampled from retail outlets in Kostanay region.

Materials and Methods

The study was conducted in the microbiological analysis laboratory of the Scientific Research Institute of Applied Biotechnology at A. Baitursynuly Kostanay Regional University. A total of 30 samples of chilled meat, including pork (n=10), beef (n=10), and horse meat (n=10), were collected from five different retail outlets in accordance with the GOST 31904-2012 standard “Food products. Methods of sampling for microbiological tests” [12].

Samples were delivered to the laboratory in sterile containers, with temperature control maintained to preserve sample integrity for microbiological analysis. Upon arrival, the samples were processed immediately under aseptic conditions to prevent any extraneous contamination.

The microbiological methods employed in the study were conducted in accordance with the standards outlined in GOST [13-18].

For the identification of coliform bacteria, samples were placed in meat-peptone broth (FBIS SRCAMB, Obolensk, Russia), followed by subculturing onto Endo agar and TBX agar (CHROMagar, Paris, France) and incubated at 37 °C for 24 hours. Colonies with characteristic morphology were subjected to further biochemical analysis.

For *S. aureus*, yolk-salt agar was used, with incubation at 37 °C for 24 hours, and then at room temperature for an additional 24 hours, with confirmatory tests based on coagulase activity.

Detection of *Salmonella* spp. included pre-enrichment in buffered peptone water (FBIS SRCAMB, Obolensk, Russia), selective enrichment in Rappaport-Vassiliadis broth (FBIS SRCAMB, Obolensk, Russia), and subsequent inoculation onto Salmonella agar (CHROMagar, Paris, France).

To identify *L. monocytogenes*, samples were placed in Fraser broth (FBIS SRCAMB, Obolensk, Russia), enriched, and incubated for two days, with the first day at 30 °C and the second at 37 °C, followed by subculturing onto Listeria chromogenic agar (CHROMagar, Paris, France) and Oxford agar (Condalab, Madrid, Spain).

To determine the total microbial number (QMAFAnM), which is the quantity of mesophilic aerobic and facultative-anaerobic microorganisms, we conducted studies using the classical deep culture method in accordance with the standards outlined in GOST 26670-91 [18].

The presence of total microbial contamination was assessed by inoculating serial dilutions of the samples onto QMAFAnM media (FBIS SRCAMB, Obolensk, Russia) and incubating at 37°Cfor 24 hours, followed by an additional 24 hours at room temperature.

The arithmetic mean of the number of colonies from all cultures was calculated using the counting process results. The number of microorganisms in 1.0 g ("X") of the product was calculated using the following formula:

$$X = n \times 10^m,$$

this formula defines the variables as follows: "n" represents the number of colonies counted on the dish, while "m" denotes the number of decimal dilutions.

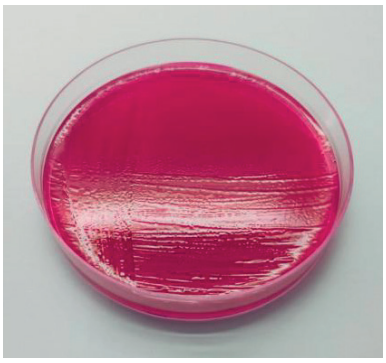
The calculation result was expressed as a number between 1.0 and 9.9×10m. The obtained number of microorganisms was then subjected to a verification process in accordance with the relevant requirements outlined in "Customs Union Technical Regulations on the safety of food products 021/2011" [19].

Results

Among the microbiological indicators, we conducted studies to determine the presence of coliform bacteria. Characteristic growth of E. coli was observed on solid differential Endo medium, forming colonies with a metallic sheen, and on CHROMagar TBX chromogenic medium, where colonies appeared blue. *Proteus spp.* colonies on Endo agar were transparent with swarming growth and a distinctive odor. Growth of *E. coli* (7 strains) and *Proteus spp*. (2 strains) was detected (Figure 1).

Growth of *E. coli* in sample No. 5 on Endo medium

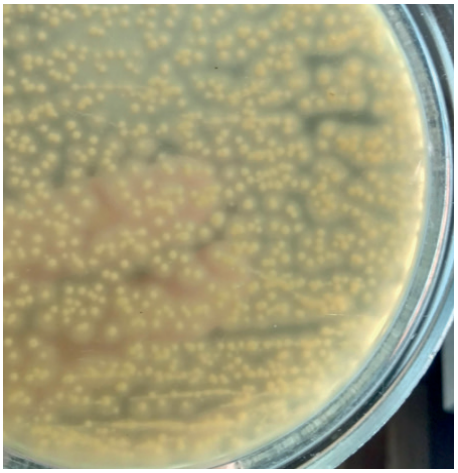
Growth of *E. coli* in sample No. 7 on CHROMagar TBX medium



Growth of *Proteus* in sample No. 2on Endo medium

Figure 1 – Microbiological investigation of coliform bacteria

Research was also carried out to detect *S. aureus*. Staphylococci demonstrated characteristic growth on yolk-salt agar, forming colonies with "a rainbow halo" (indicating lecithinase production). A total of 4 strains coagulase-positive *S. aureus* were detected (Figure 2).



Growth of *S. aureus* in sample No. 26 on yolk-salt agar

Figure 2 – Microbiological investigation of *S. aureus*

The results of these studies demonstrated that *L. monocytogenesand Salmonella spp*. bacteria were not detected in any of the tested samples.

The number of colonies grown was enumerated in the cultures. The quantity of microbial colonies was determined by counting in cultures of dilutions at 10^2, 10^3,10^4, respectively (Figure 3).

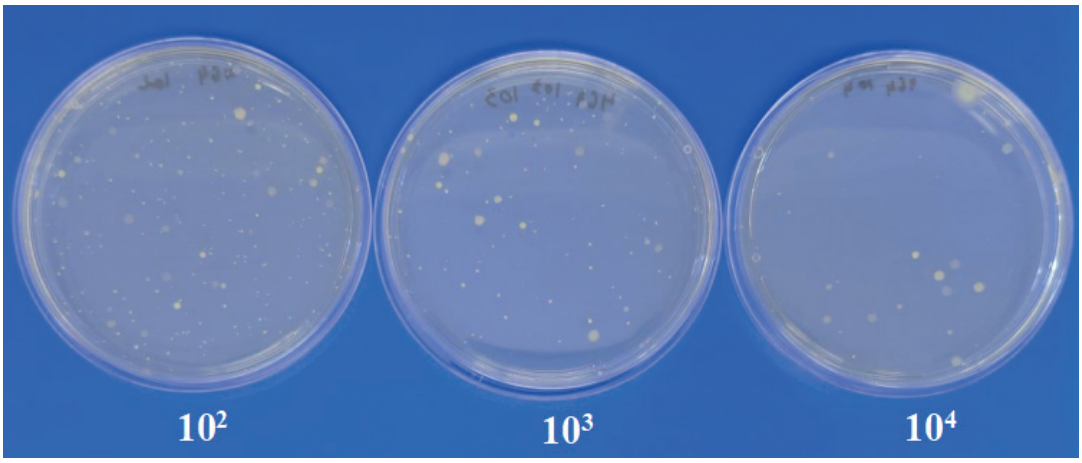


Figure 3 – Microbiological investigation QMAFAnM (dilutions: 10^2, 10^3, 10^4)

The findings of the study indicated that all 10 pork samples analysed exceeded the permissible level of QMAFAnM. Coliforms were identified in four samples (No. 2, No.8 - *Proteus spp.*, No.5, No.7 - *E. coli*). Two strains of S. aureus were isolated from samples No. 2 and No. 8. However, no evidence of *Salmonella spp.* or *Listeria spp.* bacteria was observed (Table 1).

Table 1 – Results of microbiological examination of pork

Sample №	QMAFAnM, CFU/g	Coliforms	*Salmonella spp.*	*L. monocytogenes*	*S. aureus*
1	11×10^4	–	–	–	–
2	15×10^4	+	–	–	+
3	5.4×10^4	–	–	–	–
4	7.9×10^4	–	–	–	–
5	8.2×10^4	+	–	–	–
6	8.5×10^4	–	–	–	–
7	35×10^4	+	–	–	–
8	27×10^4	+	–	–	+
9	10×10^4	–	–	–	–
10	9.7×10^4	–	–	–	–
Norma	1×10^4 CFU/g, max.	Not allowed in 0.001 g of product	Not allowed in 25 g of product	Not allowed in 25 g of product	Not allowed in 1 g of product

The analysis results of beef samples indicate that all tested meat samples exceed the permissible level of QMAFAnM. The isolation of coliform bacteria was observed in three samples (No.12, No.16, No.20 - *E. coli*). The tested samples did not show the presence of *S. aureus, Salmonella, or Listeria species* (Table 2).

Table 2 – Results of microbiological examination of beef

Sample №	QMAFAnM, CFU/g	Coliforms	*Salmonella spp.*	*L. monocytogenes*	*S. aureus*
11	5.9×10^4	–	–	–	–
12	15×10^4	+	–	–	–
13	24×10^4	–	–	–	–
14	11×10^4	–	–	–	–
15	22×10^4	–	–	–	–
16	3.4×10^4	+	–	–	–
17	1.2×10^4	–	–	–	–
18	9.7×10^4	–	–	–	–
19	6.8×10^4	–	–	–	–
20	5.6×10^4	+	–	–	–
Norma	1×10^4 CFU/g, max.	Not allowed in 0.001 g of product	Not allowed in 25 g of product	Not allowed in 25 g of product	Not allowed in 1 g of product

The data obtained indicated that nine samples of horse meat under investigation had exceeded the maximum permitted level of contaminants as defined by the QMAFAnM. In two samples, a positive reaction for the presence of coliform was identified (samples No.21 and No.29, which tested positive for E. coli). Two strains of *S. aureus* were isolated from samples No. 26 and No.28. No evidence of *Salmonella* or *Listeria bacteria* was found in any of the tested samples (Table 3).

Table 3 – Results of microbiological examination of horse meat

Sample №	QMAFAnM, CFU/g	Coliforms	*Salmonella spp.*	*L. monocytogenes*	*S. aureus*
21	12×10^5	+	–	–	–
22	5.7×10^5	–	–	–	–
23	5.2×10^5	–	–	–	–
24	5.0×10^5	–	–	–	–
25	7.3×10^5	–	–	–	–
26	11.1×10^5	+	–	–	+
27	6.3×10^5	–	–	–	–
28	10.1×10^5	–	–	–	+
29	6.6×10^5	+	–	–	–
30	7.9×10^5	–	–	–	–
Norma	1×10^4 CFU/g, max.	Not allowed in 0.001 g of product	Not allowed in 25 g of product	Not allowed in 25 g of product	Not allowed in 1 g of product

The results of the studies demonstrate notable discrepancies in QMAFAnM levels across diverse meat categories. The results indicated that the mean QMAFAnM value in pork was 13.77×10^4 CFU/g, the highest among all the meat types under investigation. This suggests that pork is more susceptible to microbial contamination than other meats. Beef demonstrated a mean QMAFAnM value of 10.46×10^4 CFU/g, which is considerably lower than pork but higher than horse meat. Horse meat exhibited the lowest level of QMAFAnM, at 7.72×10^4 CFU/g, and is the least susceptible to microbial contamination among the tested meats (Figure 3).

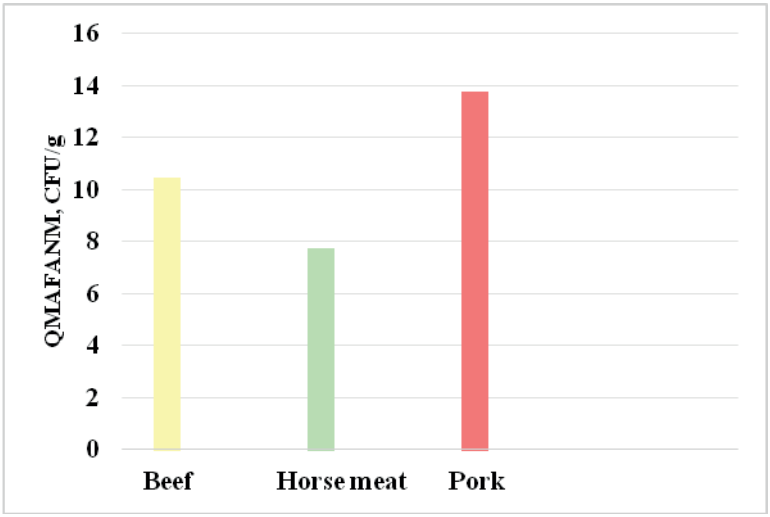


Figure 3 – Indicators of the level of microbial contamination in the studied meat samples

Discussion and Conclusion

The study's resultsrevealed notable differences in microbial contamination levels across different meat types. In all pork samples analyzed, the total microbial count (QMAFAnM) exceeded permissible limits, consistent with findings from other studies on microbial contamination in pork [20, 21]. Additionally, coliform bacteria and S.aureus were detected in pork samples, indicating potential health risks for consumers and possible lapses in hygiene protocols in the handling and processing of pork.

A similar contamination pattern was observed in the beef samples. All beef samples analyzed also had QMAFAnM levels above the acceptable standards, with coliforms present in three samples. This finding aligns with previous research [22] and may suggest deficiencies in the sanitary and hygienic processing of beef, potentially at various stages from production to storage. These issues highlight the need for more rigorous quality control measures for beef to prevent microbial contamination that could pose health risks.

The analysis of horsemeat samples showed that nine out of ten samples had QMAFAnM concentrations above permissible limits, though contamination levels were generally lower than those observed in pork and beef. Coliform bacteria and *S. aureus* were detected in two horsemeat samples, suggesting a degree of bacterial contamination but at a lower frequency than in the other meat types. This may reflect variations in the conditions under which horsemeat is handled and stored, yet underscores the importance of maintaining strict hygiene practices across all meat types.

Importantly, no samples from any of the meat types tested positive for the presence of pathogenic bacteria such as *Salmonella spp.* or *L. monocytogenes* indicating that while general bacterial contamination was an issue, there was no evidence of these specific pathogens in the samples.

To enhance the safety and quality of meat, adherence to microbiological control measures as set forth by the technical regulations of the Customs Union (021/2011,034/2013) [19, 23] is crucial. These regulations govern the production, storage, transportation, sale, and disposal of food products, ensuring safety standards are upheld at every stage in the supply chain.

The study results indicate the necessity for enhanced monitoring and regulation of the sanitary and hygienic conditions associated with the production, processing, and storage of meat of diverse types. The presence of elevated levels of QMAFAnM and the occurrence of coliform bacteria in meat products suggest potential risks to consumer health, underscoring the importance of implementing more rigorous measures to ensure food safety. Regular monitoring of microbial contamination and staff training in meat handling and storage practices are crucial strategies for reducing the risk of contamination.

Authors' Contribution

RR and AM: conceptualized and designed the study, conducted a comprehensive literature search, and analyzed the collected data. YuA, ME, and AG: carried out the research implementation and

analyzed the results. RR, ZA, and AG: performed the final editing and proofreading of the manuscript. All authors have read, reviewed, and approved the final version of the manuscript.

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