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IDENTIFICATION OF PRIORITY BACTERIAL GROUPS TO OPTIMIZE SANITARY PROCEDURES AT MEAT PROCESSING PLANTS

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Abstract

This paper presents the study results of the microbiological composition in the industrial environment at four pork slaughter and processing plants (MPPs). The sample included plants with various production problems and different process features. The purpose of this study was to determine the priority bacterial groups typical for all studied plants, as well as to identify specific microorganisms associated with the individual characteristics of each plant. Representatives of Pseudomonas, Candida, and Escherichia genera dominated at all four plants, but each plant had its own unique characteristics. Thus, at MPP No. 1, where no preliminary decapitation was performed, a high level of industrial environment contamination with Escherichia genus microorganisms and pathogenic microorganisms, Salmonella spp. and Listeria monocytogenes, was observed. At MPP No. 2, which allows the acceptance of raw materials with defects, a significant counts of Staphylococcus genus microorganisms were detected. Pseudomonas, Carnobacterium, and Enterobacteriaceae genera were detected at MPPs No. 3 and No. 4, where systematic spoilage of finished products was revealed. Analysis results showed that individual technological stages and conditions at different plants create a unique environment that promotes the development of certain groups of microorganisms. The introduction of expanded microbiological monitoring, changes in technology, and the development of individual recommendations for each plant will reduce the risks of microbial contamination, improve product quality, and increase its safety for consumers.

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Introduction

Each plant has unique operating conditions, technological processes and ecological status, which affects the composition and dynamics of microflora. The use of standard indicators may overlook locally significant pathogens or opportunistic bacteria that may affect the product quality and consumer health in a given plant. Even meat processing plants of the same profile may differ in technological processes, features of slaughter and cutting, and face different problems (spoilage of finished products, ineffective disinfection). These aspects play a key role in the formation of a unique microflora inside the plant and, as a result, in the optimization and customization of sanitary procedures.

Microbiota in food production is often considered as a possible source of microorganisms that may affect the quality of meat products [1]. A number of studies have confirmed that microorganisms found in the product are often found on plant surfaces and equipment [2,3]. Regular use of sanitizers helps to eliminate microorganisms, however, when bacteria are found in biofilms or disinfectants are ineffective against certain groups of microorganisms, the risk of food contamination and outbreaks of infectious diseases increases [4].

According to the European Union's Zoonoses Report for 2022, *Salmonella* bacteria were identified in 951.590 meat and meat product samples, while *Campylobacter* bacteria were detected in 107.162 samples. In addition, *Listeria monocytogenes* was detected in 135.148 ready-to-eat (RTE) food samples [5].

In our country, product safety control is traditionally based on determining the presence of pathogenic microorganisms directly in the product. This approach allows identifying the potential risks of finished products before they are released to the market. However, this method does not cover the entire production process and does not take into account possible sources of contamination at different stages of production [6]. It is important to understand that product safety depends on many factors, from animal handling conditions to compliance with sanitary standards at each stage of raw material processing. To ensure comprehensive product safety, it is necessary to monitor the

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entire production chain, including regular analysis of the environment, equipment, surfaces and air [7].

In addition to human pathogens, other groups of bacteria that may be present in food products and are potentially dangerous to humans or affect the quality and shelf life of the product, thereby leading to economic loss and increased costs for maintaining the sanitary status of the plant [8] should also be considered. These include spoilage bacteria and industry-specific bacterial groups, emerging microorganisms, ESKAPEE, etc.

Spoilage microorganisms. Spoilage microorganisms include bacteria, fungi, and yeasts that may cause changes in the sensory properties of food products. These microorganisms may be present even under strict sanitary conditions, so monitoring them is necessary to prevent premature deterioration of product quality. The most common bacteria that cause spoilage of chilled beef and pork during aerobic storage include *Brochothrix thermosphacta, Carnobacterium* spp., *Enterobacteriaceae* family, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Weissella* spp. The metabolic processes of these microorganisms may cause defects such as sour taste, color changes, gas formation, mucus formation, and decreased pH [9].

Industry-specific bacterial groups. Each food industry has its own specific bacterial species. For example, in ready-to-eat food production, special attention is paid to bacteria such as *Listeria monocytogenes* causing listeriosis in humans [10]. In fish processing plants, emphasis is placed on preventing the growth of *Vibrio* spp. and *Aeromonas hydrophila*, which are capable of causing foodborne illnesses [11].

Emerging microorganisms. Emerging microorganisms are new or previously little-known pathogens that are becoming increasingly important due to changes in agricultural practices, food processing, and globalization of trade. They are of serious hazard to consumer health and require special attention from the food industry. Examples of emerging pathogens include *Escherichia coli* O157: H7 and *Campylobacter jejuni* causing serious food poisoning [12].

ESKAPE group. According to the US Centers for Disease Control (CDC), more than 2 million cases of illness and about 23 thousand deaths are associated with ESKAPE pathogens every year [13]. To attract the attention of the scientific community, CDC introduced the term "ESKAPE pathogens", which includes six types of microorganisms that are highly resistant to antibiotics and may cause hospital-acquired infections [14].

There is information about seven types of pathogens with high resistance to antibiotics, including *Escherichia coli*; in this case the abbreviation ESKAPEE is used [15]. Although these microorganisms are more often associated with medical institutions, they are also found at food plants. This is due to the uncontrolled use of antibiotics in animal husbandry and veterinary medicine, which leads to the emergence of resistant bacteria in livestock. Resistant bacteria can enter the food chain through the use of contaminated meat, milk, eggs and their processed products [16]. For example, *E. coli* and *K. pneumoniae*, commonly found in farm animals, may acquire resistance genes and transmit them to humans through the consumption of meat products [16].

Sanitary and microbiological study of microbial contamination of industrial objects at food plants. In the Russian Federation, bacteriological study of industrial objects at a food plant involves the determination of only three indicators: coliforms, *S. aureus* and total microbial count (TMC)¹.

These groups of microorganisms are considered universal indicators of food safety and production sanitation. However, such approaches have limitations. Firstly, they are usually developed on the basis of general standards and recommendations applicable to all food industry enterprises, without taking into account the specifics of a particular production. This may lead to the fact that the studied indicators do not reflect the real microbiological state of a particular plant.

Thus, a generalized approach to defining a limited group of microorganisms does not always provide accurate information on the actual conditions of microorganism circulation on a specific plant [17]. To obtain more reliable data, it is necessary to conduct individual microbiological studies that take into account the characteristics of each individual production, identify priority bacterial groups and select effective disinfectants based on these data.

The purpose of this study was to determine the priority bacterial groups typical for pork slaughter and processing plants (n=4), as well as to identify specific microorganisms associated with the individual characteristics of each plant.

Objects and methods

Objects

The objects of the study were swabs (n=113) collected in different production areas at pork slaughter and processing plants (n=4). When collecting swabs, we used the principles of environmental monitoring and collected swabs both from surfaces in contact with food products and from remote abiotic objects [7].

Sampling of swabs was carried out in key areas of the production cycle: slaughterhouse, primary processing shop, cold rooms, and semi-finished products shop. The number of swabs collected in each area is presented in Table 1.

¹MR4.2.0220-20. 4.2. Monitoring methods. Biological and microbiological factors. Methods for sanitary and bacteriological research of microbial contamination of industrial objects. Methodological recommendations (approved by the Chief State Sanitary Doctor of the Russian Federation on 04.12.2020) Retrieved from https://docs.cntd.ru/document/573595605 Accessed April 11, 2025

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	MPP No. 1	MPP No. 2	MPP No. 3	MPP No. 4		
Slaughterhouse	<i>n</i> = 8	n = 7	n = 7	n = 7		
Primary processing shop (boning and trimming)	<i>n</i> =7	<i>n</i> =7	<i>n</i> =7	<i>n</i> =7		
Cold rooms	n = 7	n = 7	n = 7	n = 7		
Semi-finished products shop	n = 7	n = 7	n = 7	n = 7		

Table 1. Number of swabs collected in each area of MPP

The sample included plants with various production problems and process features. MPP No. 1 slaughtered animals without prior decapitation, MPP No. 2 accepted raw materials with defects (abscesses), while MPP No. 3 and No. 4 had problems with shelf life and deterioration in the quality of finished products.

Methods

Sampling the swabs from the objects of the industrial environment

Swabs from the industrial objects at pork processing plants were collected using a sponge with a neutralizer (3M Hydra-Sponge, USA) from 100 cm², and in case of hard-to-reach places, using sterile cotton swabs with lethin broth ($3M^{Te}$ Swab-Sampler, USA). The resulting samples were incubated in Binder thermostat at 30 °C for 72 hours. After incubation, colonies with different morphology were selected from Petri dishes and identified using mass spectrometric analysis on Autof MS1000 MALDI-TOF device (Autobio, China).

An aliquot of 100 μ l of liquid was taken from the bag with the sponge, which was distributed using a sterile spatula onto the surface of non-selective TSA agar (Oxoid, UK) in a Petri dish. After 72 hours of incubating the inoculations at a temperature of 24 °C, colonies were selected for species identification.

Detection of pathogenic microorganisms in the objects of the industrial environment

To detect *Listeria monocytogenes*, semi-concentrated Fraser broth was used and incubated in a thermostat (Binder, Germany) at a temperature of 30.0 ± 1.0 °C for 24 hours. To detect *Salmonella* spp., buffered peptone water was used as a diluent and incubated at a temperature of 37 ± 1.0 °C for 18 to 24 hours. To detect *Campylobacter* spp., an enrichment medium for *Campylobacter* (3M, USA) was used and incubated at a temperature of 41.5 ± 1.0 °C for 22 to 26 hours. Further studies were carried out in accordance with GOST 31659 (ISO 6579: 2002)², GOST 32031-2012³ and GOST ISO 10272-1-2013⁴.

Microorganism species identification by mass spectrometry

Species identification of the isolated colonies was performed on Autof MS1000 MALDI-TOF mass spectrometer (Autobio Diagnostics, China). For this, the bacterial mass of the colonies was applied to a plate and dried at room temperature. Then, 1.2 μ l of formic acid was applied to each well with the dried bacterial mass for 10 min, dried, 1.2 µl of HCCA matrix (a-cyano-4-hydroxycinnamic acid, 99%) was applied and dried again. The MALDI target was placed in the device and the equipment for microorganism identification was launched using the FlexControl software (spectra acquisition). The obtained results were analyzed using the software: if the value was below 6.0, the result was considered unreliable and was not used in further work. The result was considered reliable and taken into account at values of 6.0 to 9.0 at the genus level, and at values of 9.0 to 9.5 at the species level.

Results and discussion

Since all four studied plants specialized in pork slaughter and processing, it was reasonable to assume that they shared similar production conditions, such as the use of the same type of raw material (pork) and the same technological stages: slaughter, bleeding and subsequent cutting of carcasses. These common factors create conditions for the formation of stable microflora specific to this type of meat processing. Consequently, it could be expected that certain genera of microorganisms would be present on all four plants, playing a key role in the formation of the general microbiota of the industrial environment (Figure 1).

As a result of the microflora analysis at pork slaughter and processing plants, about 47 genera of microorganisms were found, of which 24 most common are presented in Figure 1. Genera composed less than 1% are not shown in Figure 1.

The most common genera were *Pseudomonas* (26.3%), Escherichia (8.8%) and Candida (8.3%). Bacteria of Pseudomonas genus were predominant and accounted for 26.30% of the total counts of identified microorganisms. These microorganisms are considered to be one of the main causes of spoilage of meat, fruits and even beverages packaged in an aerobic environment [18]. In a study by Chinese scientists on microflora responsible for the spoilage of chilled pork, it was found that bacteria of Pseudomonas genus also accounted for the majority of the identified microorganisms. The average relative abundance of Pseudomonas in the product was 24.77%, and the maximum abundance reached 44.43% on the seventh day of storage [19]. This may be due to the high adaptability of pseudomonads to various conditions, including low temperatures, and the ability to effectively use available nutritional sources, which makes them dangerous for many food products [20]. There is evidence that the nature of spoilage may depend on both the species and the strain of Pseudomonas [21]. In a study conducted jointly by Italian

² GOST 31659 (ISO 6579: 2002) "Food products. Method for the detection of Salmonella spp." Retrieved from https://docs.cntd.ru/document/1200098239 Accessed April 11, 2025

³ GOST 32031-2012 "Food products. Methods for detection of Listeria monocytogenes" Retrieved from https://docs.cntd.ru/document/1200105310 Accessed April 11, 2025 ⁴ GOST ISO 10272-1-2013 "Microbiology of food and animal feeding stuffs.

⁴ GOST ISO 10272-1-2013 "Microbiology of food and animal feeding stuffs. Methods for detection and enumeration of Campylobacter spp. Part 1. Detection method" Retrieved from https://docs.cntd.ru/document/1200103500 Accessed April 11, 2025

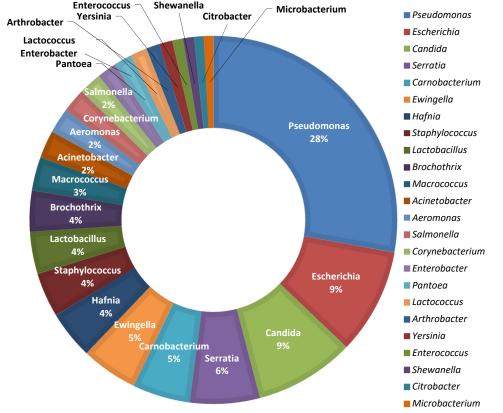


Figure 1. Predominant genera of microorganisms in the industrial environment of pork slaughter and processing plants

and American scientists, the diversity of Pseudomonas populations found during the processing of meat and dairy products was analyzed. It was found that the most common oligotypes were those belonging to the species P. fragi and P. fluorescens [21]. In our study at pork slaughter and processing plants, the diversity of microorganisms of this genus was represented by 40 species, of which 20.1% were Pseudomonas brenneri, followed by Pseudomonas fragi and Pseudomonas gessardi (9.3%), Pseudomonas libanensis (7.2%), Pseudomonas tolaasi (7.1%), and Pseudomonas fluorescens (5.5%). The data obtained in our study, as well as the results of other scientific works, confirm the widespread occurrence of Pseudomonas at meat processing plants. However, despite this, monitoring of these bacteria when taking swabs from the surfaces of equipment is not carried out. Given their high resistance to low temperatures and the ability to cause spoilage of products, regular analysis of swabs from equipment for the presence of pseudomonads would allow for their timely detection and the adoption of appropriate measures for disinfection and improvement of sanitary procedures.

The high level of *Escherichia* spp. occurrence (8.80%) indicates significant risks associated with food spoilage and potential risks to consumer health, since the vast majority of isolated strains belonged to *Escherichia coli* species. It should be remembered that pathogenic strains such as enterohemorrhagic *Escherichia coli* (EHEC) may cause severe food poisoning, accompanied by hemorrhagic colitis, which is life-threatening. The main source of *Escherichia coli* at pork processing plants is animal intestines, so these microorganisms may serve as an indicator of fecal

contamination, which indicates the possible presence of other pathogenic microorganisms inhabiting animal intestines such as *Salmonella* or *Campylobacter*. It is worth noting that the distribution of pathogenic bacteria at different plants was not uniform. The largest counts of them were found on the plant, where pigs were slaughtered without prior decapitation.

The presence of yeasts of *Candida* species is also significant as these microorganisms may cause food spoilage and are of potential hazard to consumer health. *Candida* yeasts are important microbiological agents that contribute to problems in food production, especially in the context of meat processing, including pork. These microorganisms have the ability to proliferate under conditions of low pH, moderate temperatures and the presence of key nutrients such as glucose and amino acids, which determines their role as potential spoilage agents in meat products. They tend to adhere to the surfaces of equipment used for meat processing, forming biofilms that are resistant to standard sanitation and disinfection methods [22]. This phenomenon may lead to recurrent contamination of the manufactured products.

The next most common genera were *Serratia* (5.8%), *Carnobacterium* (4.8%), and *Ewingella* (4.7%). These microorganisms are also gram-negative bacteria and may be involved in food spoilage processes and also may be indicators of environmental contamination. Other genera present in smaller quantities include *Staphylococcus* (3.7%), *Lactobacillus* (3.5%), and *Brochothrix* (3.4%). *Staphylococcus* spp. are potential pathogens that may cause food poisoning, while *Lactobacillus* and *Brochothrix* may be associated with fermentation and food spoilage processes. The presence of such microorganisms as *Carnobacterium* (4.80%), *Lactobacillus* (3.50%), and *Brochothrix* (3.40%) may indicate potential problems associated with spoilage. These bacteria are known to affect the sensory properties of meat, making their presence undesirable during the processing and storage of meat products. *Carnobacterium* is a genus of Gram-positive bacteria that is often found in chilled food products, including meat. They may cause changes such as unpleasant odor and taste, as well as deterioration in the appearance of the product. These bacteria are able to actively grow even at low temperatures, which makes them especially dangerous in refrigerated storage conditions [23].

Lactobacillus is another group of bacteria that can be present in meat products. Although some lactobacilli species are used in food industry for fermentation, excessive amounts of these microorganisms may alter the sensory characteristics of meat (acidity), which in turn affects its quality and shelf life. In recent years, there has been considerable interest in the scientific community in exploring the possibilities of using lactic acid microorganisms as bioprotectants to combat spoilage bacteria such as *Pseudomonas*. Studies have confirmed that difficulties arise when attempting to transfer *in vitro* data into industrial conditions due to interactions between bacteria, antimicrobials, and food matrix structures [24].

Brochothrix is a bacterium known for its effect on the aroma and appearance of meat. It is capable of producing specific compounds that cause unpleasant odors and tastes, which reduces the quality of the final product [25].

In our country, monitoring of the sanitary and microbiological state of production is reduced to taking swabs from the hands of personnel, equipment surfaces, and inventory in contact with products. Abiotic objects not contacting with products are ignored. In addition, swabs are sampled before work or after proper surface treatment. In other words, the purpose of taking swabs is to control disinfection, and not to identify microorganisms circulating at the plant. Given the prevalence of *Pseudomonas*, yeasts, and lactic acid microorganisms in the industrial environment, it is important to include in the monitoring program not only traditional indicators, but also other groups that are significant for the plant. In addition, to obtain an objective picture of the microbial composition, it is recommended to collect swabs not only after disinfection, but also during the work process. This will reveal the real degree of contamination and circulation of microorganisms at the plant, which will help to more effectively develop strategies for the prevention and management of microbiological safety risks [26].

After completing the analysis of the common microbiota at the four studied plants, a detailed analysis of the individual microflora at each plant was carried out, taking into account the features of technological processes and specific problems in production. This approach allowed to better understand the structure of the microbiological community at each plant and identify the features of microorganism distribution depending on the nature of production operations (Figures 2 to 5).

Figure 2 shows the microflora at MPP No. 1, where the technology of slaughtering animals without preliminary decapitation is used. This plant showed a high level of bacteria of *Escherichia* genus, which constituted the largest percentage among all identified strains. Such growth indicates a direct connection between the slaughter technology used and an increased risk of microbiological contamination of both the industrial objects and the finished product. As described earlier, a high level of *Escherichia* (a sanitary indicator microorganism) serves as an indicator of the possible presence of other microorganisms that are part of normal gastrointestinal microflora of animals, including potentially pathogenic species. This fact was confirmed in this study: it was MPP No. 1 that had the highest level of pathogenic microorganisms in the industrial objects.

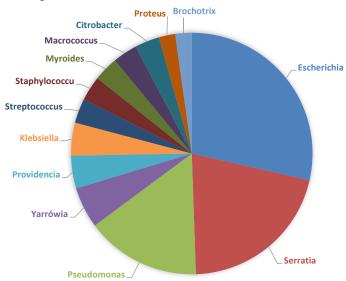


Figure 2. Microflora of the industrial environment at MPP No. 1

Figure 3 shows the microflora at MPP No. 2, where raw materials with defects (abscesses) were accepted, which subsequently affected the results of microbiological analysis. The high percentage of staphylococci detected (14.5%)

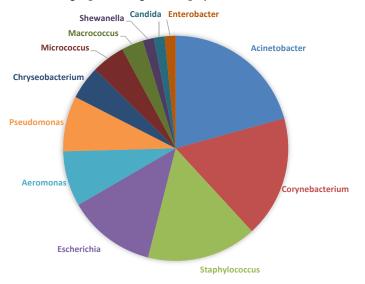
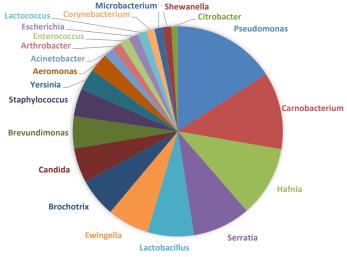
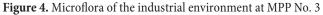


Figure 3. Microflora of the industrial environment at MPP No. 2





of all identified strains) in swabs confirmed the contamination of the industrial objects due to the use of low-quality raw materials. One of the main causes of abscess development is Staphylococcus aureus, which is highly virulent and can penetrate deep into the body tissues. Staphylococcus aureus is also a member of pathogenic group known as ESKAPE, which is characterized by multidrug resistance, making the treatment of infections in humans extremely difficult [27,28]. It is worth noting that Staphylococcus aureus is very dangerous not only due to its multiple antibiotic resistance, but also due to its ability to produce enterotoxins (A, B, C, D, E), which may cause severe food poisoning associated with the consumption of raw, undercooked or improperly processed products [29]. In our previous study, the microbial composition of minced meat intended for the production of dry-cured sausages was analyzed. During the study, 2 enterotoxigenic strains of Staphylococcus aureus were identified [30]. The detection of such strains emphasizes the importance of strict quality control of raw materials and compliance with sanitary and hygienic standards at all stages of production.

At MPP No. 2, the dominant genus of microorganisms was *Acinetobacter*. A representative of this genus, *Acinetobacter baumannii*, is also included in the ESKAPE group of pathogens. The risk of foodborne ESKAPEE infections is particularly high for hospital patients, as these microorganisms may be spread through hospital kitchens [31]. There is a report that strains of *Acinetobacter baumannii* causing enterogenous sepsis have been isolated from hospital kitchens in Portugal and Brazil [32].

At meat processing plants No. 3 and No. 4, problems related to spoilage and shelf life of finished products were observed. These difficulties negatively affected the quality of the products, and also led to financial losses and a decrease in consumer confidence (Figures 4 and 5). Bacterial groups responsible for spoilage of chilled meat and meat products are usually *Pseudomonas* spp. and *Brochothrix thermosphacta* [33]. Microbiological analysis performed at these two plants showed that the dominant group of microorganisms at plant No. 3 was *Pseudomonas* spp., fol-

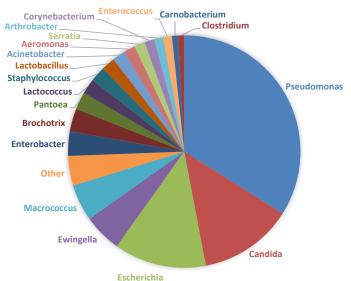


Figure 5. Microflora of the industrial environment at MPP No. 4

lowed by *Carnobacterium* spp. (12%). At MPP No. 3, the share of *Brochothrix* genus microorganisms was 6%, and at MPP No. 4 it was 2%. In addition to *Pseudomonas* spp., at MPP No. 3, high levels of other *Enterobacteriaceae* family representatives were revealed: *Hafnia* genus amounted to 11%, *Serratia* genus amounted to 9%, and *Ewingella* genus amounted to 6.5%. It is known that enterobacteria are considered indicator bacteria of the sanitation state of the production process, since their presence may indicate poor compliance with hygienic standards, violation of raw material processing technologies, or improper equipment disinfection [34].

Hafnia spp. are facultative anaerobes, motile due to flagella. The best-known representative of this genus is *Hafnia alvei*. These bacteria live in soil, water, food products and the intestines of animals. They rarely cause diseases in humans, but may sometimes cause urinary infections, gastrointestinal infections and even sepsis in patients with weakened immunity [35,36]. In healthy people, they most often act as saprophytes.

Serratia spp. is a genus of gram-negative bacteria that can play a significant role in the process of meat spoilage. These microorganisms are capable of producing proteolytic enzymes destroying proteins that are part of muscle tissue. Proteolysis leads to changes in the structure of meat, unpleasant odor and deterioration of the sensory properties of the product [37].

Ewingella spp. is a genus of bacteria from *Enterobacteriaceae* family, which is represented by only species, *Ewingella americana*. They are facultative anaerobes and have low motility [38]. *Ewingella americana* is found in the environment, particularly in soil and water. It rarely causes diseases in humans, although cases of bacteremia and septicemia have been reported, especially in immunocompromised individuals. Its clinical significance remains poorly understood. This genus of bacteria is rarely found in meat products, and its presence may be due to contamination from equipment, water, or other external sources [39].

Pl	lant	process failure/production problem	Microbiological consequences	Problem solution Changing slaughter technology (preliminary decapitation). Strengthening sanitation of surfaces and equipment. Introducing tests for enterohemorrhagic strains of <i>E. coli</i> .		
MPP	9 No. 1	Slaughter without prior decapitation	Growth of <i>Escherichia</i> spp.			
МРР	P No. 2	Acceptance of raw materials with defects (abscesses)	Growth of <i>Staphylococcus</i> spp. and <i>Corynebacterium</i> spp.	Separating defective and non-defective carcass processing lines. Strengthening sanitary procedures.		
MPP	P No. 3	Systematic spoilage of finished products	Growth of psychrotrophs (<i>Pseudomonas</i> spp., <i>Carnobacterium</i> spp.)	Using disinfectants effective against biofilms; microbiological control of water.		
МРР	P No. 4	Systematic spoilage of finished products	Growth of <i>Pseudomonas</i> spp., <i>Candida</i> spp.	Using disinfectants effective against yeasts, as well as biofilms; microbiological control of water.		

Table 2. Example of in	ndividual recommend	lations for improvi	ing sanitation at mea	t processing plants

At MPP No. 4, *Ewingella* genus microorganisms were also detected, which, along with representatives of *Pseudomonas, Candida, Escherichia,* and *Macrococcus* genera, formed a stable microflora. *Pseudomonas* genus microorganisms accounted for 32% of all identified strains. The shares of *Candida* spp., *Escherichia* spp., *Ewingella* spp., and *Macrococcus* spp. were 12.3%, 12.1%, 5%, and 4.8%, respectively. *Macrococcus* genus microorganisms are often associated with the skin of animals or personnel. Their presence may be due to insufficient processing of carcasses or violation of sanitation processes [40].

Thus, the microbiological profile of MPP No. 4 reflected both the bacteria typical for meat processing plants (*Pseudomonas* spp., *Candida* spp., *Escherichia* spp.) and the unique features associated with the presence of *Ewingella* and *Macrococcus*.

The data obtained may form the basis for developing individual recommendations for improving sanitation at each plant.

Conclusion

Meat production technology plays a key role in the formation of microbiota at a plant, since each stage of the technological process creates unique conditions that promote the growth of certain groups of microorganisms. A general trend towards the dominance of *Pseudomonas, Candida* and *Escherichia* genera was revealed at the studied pork slaughter and processing plants (n=4). However, each plant had its own unique microbiological characteristics due to the specifics of technological processes and production conditions.

Thus, at MPP No. 1, where preliminary decapitation was not carried out, a high level of industrial objects contamination with *Escherichia* and pathogenic *Salmonella* spp. and *Listeria monocytogenes* was revealed, indicating contamination with gastrointestinal microorganisms. At MPP No. 2 using raw materials with abscesses, significant counts of *Staphylococcus* genus microorganisms were found. At MPP No. 3 and No. 4, where spoilage of finished products was observed, *Pseudomonas* spp., *Carnobacterium* spp. and *Brochothrix* spp. were identified.

Given the prevalence of *Pseudomonas*, yeasts (*Candida*), and representatives of *Enterobacteriaceae* family in the industrial environment of all studied plants, it is important to expand the microbiological monitoring program. In addition to traditional indicators (total microbial count, coliforms, *Staphylococcus aureus*), it is necessary to include priority groups of microorganisms for each plant, such as *Pseudomonas, Candida, Enterobacteriaceae*, as well as pathogens (*Salmonella* spp., *Listeria monocytogenes*). This will allow for more accurate assessment of microbial contamination risks and timely measures to eliminate them.

The data obtained may form the basis for developing individual recommendations for improving sanitation at each plant. For example, at MPP No. 1, it is necessary to strengthen control over cutting carcasses and preliminary decapitation in order to minimize contamination with gastrointestinal microorganisms. At MPP No. 2, it is important to pay attention to the quality of incoming raw materials and their preprocessing. At MPP No. 3 and No. 4, it is necessary to optimize the product cooling and storage system, as well as strengthen equipment disinfection measures.

Thus, the implementation of advanced microbiological monitoring and the development of individual recommendations for each plant will reduce the risks of microbiological contamination, improve product quality and increase its safety for consumers.

REFERENCES

- Hultman, J., Rahkila, R., Ali, J., Rousu, J., Björkroth, K. J. (2015). Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuumpackaged cooked sausages. *Applied and Environmental Microbiology*, 81(20), 7088–7097. https://doi.org/10.1128/ AEM.02228-15
- Calasso, M., Ercolini, D., Mancini, L., Stellato, G., Minervini, F., Di Cagno, R. et al. (2016). Relationships among house, rind and core microbiotas during manufacture of traditional Italian cheeses at the same dairy plant. *Food Microbiology*, 54, 115–126. https://doi.org/10.1016/j.fm.2015.10.008
- 3. Stellato, G., De Filippis, F., La Storia, A., Ercolini, D. (2015). Coexistence of lactic acid bacteria and potential spoilage mi-

crobiota in a dairy processing environment. *Applied and Environmental Microbiology*, 81(22), 7893–7904. https://doi.org/10.1128/AEM.02294-15

- Alvarez-Ordóñez, A., Coughlan, L. M., Briandet, R., Cotter, P. D. (2019). Biofilms in food processing environments: Challenges and opportunities. *Annual Review of Food Science and Technology*, 10(1), 173–195. https://doi.org/10.1146/annurevfood-032818-121805
- EFSA and ECDC. (2023). The European Union one health 2022 zoonoses report. EFSA Journal, 21(12), Article e8442. https://doi.org/10.2903/j.efsa.2023.8442
- Thakali, A., MacRae, J. D. (2021). A review of chemical and microbial contamination in food: What are the threats to a circular food system? *Environmental Research*, 194, Article 110635. https://doi.org/10.1016/j.envres.2020.110635
- Mota, J. D. O., Boué, G., Prévost, H., Maillet, A., Jaffres, E., Maignien, T. et al. (2021). Environmental monitoring program to support food microbiological safety and quality in food industries: A scoping review of the research and guidelines. *Food Control*, 130, Article 108283. https://doi. org/10.1016/j.foodcont.2021.108283
- Fusco, V., Abriouel, H., Benomar, N., Kabisch, J., Chieffi, D., Cho, G. S. et al. (2018). Opportunistic food-borne pathogens. Chapter in a book: Food safety and preservation. Academic Press, 2018. https://doi.org/10.1016/B978-0-12-814956-0.00010-X
- Casaburi, A., Piombino, P., Nychas, G. J., Villani, F., Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology*, 45(Part A), 83–102. https://doi.org/10.1016/j.fm.2014.02.002
- Kurpas, M., Wieczorek, K., Osek, J. (2018). Ready-to-eat meat products as a source of *Listeria monocytogenes*. *Journal* of Veterinary Research, 62(1), 49–55. https://doi.org/10.2478/ jvetres-2018-0007
- 11. Zaher, H. A., Nofal, M. I., Hendam, B. M., Elshaer, M. M., Alothaim, A. S., Eraqi, M. M. (2021). Prevalence and antibiogram of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* in the flesh of Nile tilapia, with special reference to their virulence genes detected using multiplex PCR technique. *Antibiotics*, 10(6), Article 654. https://doi.org/10.3390/antibiotics10060654
- Bai, R., Wang, X., Zou, Z., Zhou, W., Tan, C., Cao, Y. et al. (2024). Limited transmission of carbapenem-resistant *Klebsiella pneumoniae* between animals and humans: A study in Qingdao. *Emerging Microbes and Infections*, 13(1), Article 2387446. https://doi.org/10.1080/22221751.2024.2387446
- 13. Center for disease control and prevention. (2013). Antibiotic resistance threats in the United States, 2013. Retrieved from http://www.cdc.gov.abcs/index.html Accessed March 10, 2025.
- De Oliveira, D. M., Forde, B. M., Kidd, T. J., Harris, P. N., Schembri, M. A., Beatson, S. A. et al. (2020). Antimicrobial resistance in ESKAPE pathogens. *Clinical Microbiology Reviews*, 33(3), Article e00181–19. https://doi.org/10.1128/ cmr.00181-19
- Oyenuga, N., Cobo-Díaz, J. F., Alvarez-Ordóñez, A., Alexa, E.-A. (2024). Overview of antimicrobial resistant ES-KAPEE pathogens in food sources and their implications from a one health perspective. *Microorganisms*, 12(10), Article 2084. https://doi.org/10.3390/microorganisms12102084
- Economou, V., Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance*, 8, 49–61. https://doi.org/10.2147/IDR.S55778
- 17. Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F., Ercolini, D. (2016). Overlap of spoilage-associated microbiota between meat and the meat processing environ-

ment in small-scale and large-scale retail distributions. *Applied and Environmental Microbiology*, 82(13), 4045–4054. https://doi.org/10.1128/AEM.00793-16

- Li, N., Zhang, Y., Wu, Q., Gu, Q., Chen, M., Zhang, Y. et al. (2019). High-throughput sequencing analysis of bacterial community composition and quality characteristics in refrigerated pork during storage. *Food Microbiology*, 83(10), 86–94. http://doi.org/10.1016/j.fm.2019.04.013
- Wang, X., Deng, Y., Sun, J., Ding, Y., Liu, Y., Tian, T. (2021). Unraveling characterizations of bacterial community and spoilage profiles shift in chilled pork during refrigerated storage. *Food Science and Technology*, 42, Article e80321. https://doi.org/10.1590/fst.80321
- 20. De Filippis, F., La Storia, A., Villani, F., Ercolini, D. (2013). Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. *PLoS One*, 8(7), Article e70222. https://doi.org/10.1371/journal. pone.0070222
- Stellato, G., Utter, D. R., Voorhis, A., De Angelis, M., Eren, A. M., Ercolini, D. (2017). A few *Pseudomonas* oligotypes dominate in the meat and dairy processing environment. *Frontiers in Microbiology*, 8, Article 264. https://doi. org/10.3389/fmicb.2017.00264
- Daneshnia, F., de Almeida Júnior, J. N., Ilkit, M., Lombardi, L., Perry, A. M., Gao, M. et al. (2023). Worldwide emergence of fluconazole-resistant Candida parapsilosis: Current framework and future research roadmap. *The Lancet Microbe*, 4(6), e470-e480. https://doi.org/10.1016/S2666-5247(23)00067-8
- 23. de Andrade Cavalari, C. M., Imazaki, P. H., Pirard, B., Lebrun, S., Vanleyssem, R., Gemmi, C. et al. (2024). Carnobacterium maltaromaticum as bioprotective culture against spoilage bacteria in ground meat and cooked ham. *Meat Science*, 211, Article 109441. https://doi.org/10.1016/j.meatsci.2024.109441
- Marcelli, V., Osimani, A., Aquilanti, L. (2024). Research progress on the use of lactic acid bacteria as natural bio-preservatives against Pseudomonas spp. in meat and meat products: A review. *Food Research International*, 196, Article 115129. https://doi.org/10.1016/j.foodres.2024.115129
- 25. Fang, J., Feng, L., Lu, H., Zhu, J. (2022). Metabolomics reveals spoilage characteristics and interaction of *Pseudomonas lundensis* and *Brochothrix thermosphacta* in refrigerated beef. *Food Research International*, 156, Article 111139. https://doi.org/10.1016/j.foodres.2022.111139
- 26. 3M (2019). Environmental monitoring handbook for the food and beverage industries- terms and definitions (1st ed.), Cornell University. Retrieved from https://www.idfa. org/wordpress/wp-content/uploads/2020/03/3m-environmental-monitoring-handbook-09-2019.pdf Accessed May 16, 2025.
- 27. Gebremedhin, E. Z., Ararso, A. B., Borana, B. M., Kelbesa, K. A., Tadese, N. D., Marami, L. M. et al. (2022). Isolation and identification of Staphylococcus aureus from milk and milk products, associated factors for contamination, and their antibiogram in Holeta, Central Ethiopia. *Veterinary Medicine International*, 2022(1), Article 6544705. https://doi. org/10.1155/2022/6544705
- Wu, S., Huang, J., Wu, Q., Zhang, J., Zhang, F., Yang, X. (2018). Staphylococcus aureus isolated from retail meat and meat products in China: Incidence, antibiotic resistance and genetic diversity. *Frontiers in Microbiology*, 9, Article 2767. https://doi.org/10.3389/fmicb.2018.02767
- 29. Simmons, C. K., Wiedmann, M. (2018). Identification and classification of sampling sites for pathogen environmental monitoring programs for *Listeria monocytogenes*: Results from an expert elicitation. *Food Microbiology*, 75, 2–17. https://doi.org/10.1016/j.fm.2017.07.005

- Bataeva, D.S., Minaev, M. Yu., Makhova, A.A. (2016). Identification of enterotoxigenic staphylococci in meat raw materials. *Theory and Practice of Meat Processing*, 1(4), 19–27. https://doi.org/10.21323/2414-438X-2016-1-4-19-27 (In Russian)
- Pérez-Rodríguez, F., Taban, B. M. (2019). A state-of-art review on multi-drug-resistant pathogens in foods of animal origin: Risk factors and mitigation strategies. *Frontiers in Microbiology*, 10, Article 2091. https://doi.org/10.3389/ fmicb.2019.02091
- Malta, R. C. R., Ramos, G. L. de. P. A., Nascimento, J. D. S. (2020). From food to hospital: We need to talk about *Acinetobacter* spp. *Germs*, 10(4), 210–217. https://doi/10.18683/ germs.2020.1207
- Casaburi, A., Piombino, P., Nychas, G. -J., Villani, F., Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology*, 45(Part A), 83–102. https://doi.org/10.1016/j.fm.2014.02.002
- 34. Mladenović, K. G., Grujović, M. Ž., Kiš, M., Furmeg, S., Tkalec, V. J., Stefanović, O. D. et al. (2021). Enterobacteriaceae in food safety with an emphasis on raw milk and meat. Applied Microbiology and Biotechnology, 105, 8615–8627. https://doi.org/10.1007/s00253-021-11655-7
- 35. Cutuli, S. L., De Maio, F., De Pascale, G., Grieco, D. L., Monzo, F. R., Carelli, S. et al. (2021). COVID-19 influences lung microbiota dynamics and favors the emergence of rare infectious diseases: A case report of *Hafnia Alvei* pneu-

monia. Journal of Critical Care, 64, 173-175. https://doi. org/10.1016/j.jcrc.2021.04.008

- 36. Casanova-Román, M., Sanchez-Porto, A., Casanova-Bellido, M. (2004). Late-onset neonatal sepsis due to hafnia alvei. Scandinavian Journal of Infectious Diseases, 36(1), 70–71. https://doi.org/10.1080/00365540310017375
- 37. Lee, H. S., Kwon, M., Heo, S., Kim, M. G., Kim, G. -B. (2017). Characterization of the biodiversity of the spoilage microbiota in chicken meat using next generation sequencing and culture dependent approach. *Food Science of Animal Resources*, 37(4), 535–541. https://doi.org/10.5851/kosfa.2017.37.4.535
- 38. Hamidizade, M., Taghavi, S. M., Moallem, M., Aeini, M., Fazliarab, A., Abachi, H. et al. (2023). *Ewingella americana*: An emerging multifaceted pathogen of edible mushrooms. *Phytopathology**, 113(2), 150–159. https://doi.org/10.1094/ PHYTO-08-22-0299-R
- 39. Brightwell, G., Clemens, R., Urlich, S., Boerema, J. (2007). Possible involvement of psychrotolerant Enterobacteriaceae in blown pack spoilage of vacuum-packaged raw meats. *International Journal of Food Microbiology*, 119(3), 334–339. https://doi.org/10.1016/j.ijfoodmicro.2007.08.024
- 40. Keller, J. E., Schwendener, S., Neuenschwander, J., Overesch, G., Perreten, V. (2022). Prevalence and characterization of-methicillin-resistant Macrococcus spp. in food producing animals and meat in Switzerland in 2019. *Schweizer Archiv für Tierheilkunde*, 164(2), 153–164. https://doi.org/10.17236/sat00343

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