

FACTORS INFLUENCING MICROBIAL TRANSMISSION IN A MEAT PROCESSING PLANT

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Abstract

The review paper examines the main risk factors for microbial contamination of meat at different stages of its processing. Particular emphasis has been placed on primary animal processing being the most hazardous in terms of microbial contamination of meat. Carcass cross-contamination most frequently occurs during skinning and evisceration since hides and the digestive tract are the primary sources of microbial pathogens. It is necessary to observe stringent sanitary and hygienic rules when performing these operations. Continuous cold chain management along all following stages of meat processing and control of the sanitary status of cold chambers during meat storage are of extreme importance. An increase in the microbial counts due to the high number of manual operations was observed during meat cutting, boning, and trimming. Subsequent stages of meat processing, including mincing, curing, the addition of spices, also promote significant microbial growth. Strict control regarding detection of dangerous pathogens, especially *L. monocytogenes*, is needed at this stage. In general, to minimize problems linked with meat and meat product safety, it is necessary to take timely measures on sanitary treatment of meat processing facilities, including the prevention of biofilm formation.

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Introduction

The primary task of the food industry is to ensure the microbiological safety of manufactured products. Manufacturers have to prevent possible risks linked with microorganisms causing foodborne infections [1]. Dangerous microorganisms include bacteria (*Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Clostridium botulinum*, *Clostridium perfringens*, *Cronobacter sakazakii*, *Bacillus cereus*, *Shigella* spp., *Staphylococcus aureus*, *Vibrio* spp., *Yersinia enterocolitica*, and others), viruses (Hepatitis A virus and Norovirus) and parasites (*Cyclospora cayotensis*, *Toxoplasma gondii*, and *Trichinella spiralis*) [2].

Many factors affect microbial composition in food processing plants. Among them, a type of food processing plant, sanitary and hygienic conditions of the working environment, and microflora of raw materials are the main ones. The methodology of sampling and microbial identification influences the knowledge about microflora in a food processing plant. High-throughput sequencing opens wide possibilities for detecting microorganisms in food products being highly efficient and quick compared to the traditional methods [3]. There may be hundreds of different bacteria in a single food processing plant, but, as a rule, only a few bacterial species dominate. Six bacterial groups show high prevalence, such as *Pseudomonas*, *Acinetobacter*, *Enterobacteriaceae*, spore-forming bacteria, *Staphylococcus* spp. and lactic acid bacteria (LAB). Non-dominant species can account for more than 10% of the

total bacterial counts and show high species diversity, including *Aeromonas* spp., *Brochothrix* spp., *Microbacterium* spp., *Micrococcus* spp., *Neisseriaceae*, *Psychrobacter* spp., *Ralstonia* spp., *Rhodococcus* spp., *Shewanella* spp., *Sphingomonas* spp., *Stenotrophomonas* spp. and *Vibrio* spp. [4].

Meat is one of the most perishable foods since it contains substances necessary for rapid microbial growth. Causative agents of foodborne infections such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Clostridium perfringens*, *Escherichia coli*, *Yersinia enterocolitica*, *Staphylococcus aureus* can be present and grow in meat creating risks for consumers' health [5].

The rate of the microbiological changes in meat depends on the initial number of microorganisms, storage conditions (duration, temperature, relative humidity), and physicochemical properties of meat, such as pH and water activity [6]. Various technological operations (curing, heat treatment, cooling, and others) affect microbial communities' qualitative and quantitative composition in meat and production facilities. Production facilities are characterized by a specific temperature, humidity, sanitary and hygienic activities. Consequently, microbial communities composed of different species composition are formed in various production facilities. During processing, meat can be the primary source of microbial contamination or, *vice-versa*, contaminated by the personnel, technological equipment, utensils, containers, floors, walls, ventilation ducts, air and other sources of the production environment [7].

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Slaughter and primary carcass processing

There is a high probability of microbial contamination of meat already at the stage of animal slaughter and primary carcass processing. The preparation of animals for slaughter is an essential factor in the microbiological status of a processing plant [8]. The cleanliness of animals at slaughter is monitored in many countries. In the Russian Federation, this requirement is specified in TR CU034/2013¹.

The high risk of microbial contamination of carcasses occurs upon contact with the surface of the equipment or constructions. The existing conditions in production facilities (mainly temperature and humidity) promote an increase in microbial counts. Various microorganisms (typical for production facilities) can attach to the surfaces and form biofilms and, consequently, retain viability after cleaning and disinfection [9]. It is known that bacteria of the genus *Pseudomonas* can develop mono-species biofilms and multi-species biofilms with pathogenic microorganisms, for example, *L. monocytogenes*. This mutual biofilm formation ensures high resistance of *L. monocytogenes* during cleaning and disinfection of different surfaces (walls, equipment, and others) [10].

Species diversity of microorganisms varies depending on the type of the slaughter line. Bakhtiary et al. [11] conducted a comparative study of the microbiological carcass status on slaughter lines for cattle and small ruminants. *Salmonella*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas fluorescens* were revealed in all samples by the PCR method. The highest microbial diversity was found on the carcasses of small ruminants. Significant cross-contamination can be explained by their slaughter and carcass processing peculiarities, especially during skinning, evisceration, and scrapping. Carcass contamination is the highest when all these processes are performed manually [11].

The use of manual operations in skinning can negatively affect microbiological carcass parameters. A significant number of microorganisms are present on the surface of tools and employees' hands. The number of microorganisms can reach 2×10^7 CFU/cm² on the hands of workers performing skinning and from 6×10^3 to 6×10^8 CFU/cm² on knife surfaces (depending on the sanitary state of a food processing plant). In several cases, bacterial pathogens such as *Salmonella* were revealed on the surfaces of tools [8].

Microbial contamination of hides varies from 10^4 to 10^9 CFU/cm² depending on the sampling site [12]. The most contaminated hide areas are the distal leg (metacarpus) and brisket due to the extensive contact with fecally contaminated floors during the pre-slaughter phase [13]. Thereby, there is a high risk of microbial carcass contamination at the moment of hide cutting at these areas during manual pre-skinning. Several published studies showed that the highest carcass contamination was observed in the brisket area on the line of skin-opening cuts compared to bovine

rump, flank, and neck areas [14,15]. Coldwater washing of carcasses did not lead to significant changes in microbial counts at the sites contaminated after skinning [14].

Mechanical skinning positively influences a decrease in microbial carcass contamination [11]. Contamination with microorganisms occurs mainly due to their transmission to a carcass with dust and dirt at the moment of hide removal. It was found that the average total viable counts of bacteria (TVC) and *Enterobacteriaceae* counts (EC) on bovine hides were 5.0×10^6 and 2.0×10^4 CFU/cm², respectively [13]. *E. coli*, *Proteus*, *B. cereus*, *B. megaterium*, *Penicillium* spp., *Aspergillus* spp., and *Mucor* spp. prevailed among the detected microorganisms [16].

Pig slaughtering and porcine carcass processing are production processes with the highest risk of contamination with pathogenic microorganisms. The environment in production facilities quickly becomes contaminated during pig slaughter [17] — the risk of cross-contamination increases. Dangerous pathogens including *Salmonella*, *Campylobacter*, *Listeria*, *Enterococcus*, *Staphylococcus*, *Yersinia*, *E. coli* can be transferred to the carcass surface. *Salmonella*, *Campylobacter*, *Yersinia*, and *E. coli* can enter the production environment and meat from the animal gastrointestinal tract or skin [17]. *Yersinia* spp. can also be found on the porcine tongue and tonsils [18].

In modern production practice, swine processing is carried out without skinning. To reduce microbial contamination of surfaces, porcine skin is washed with warm water leading to an improvement in the sanitary condition of animals and reduces microbiological risks in production [17].

Bleeding of pigs is accompanied by damage of carcass integrity resulting in carcass contamination with microorganisms, including pathogens. There is a high risk of *Salmonella* contamination at this stage [17].

Scalding of pigs is performed at a temperature of 62–70 °C for several minutes. This stage can improve microbiological indices of meat carcasses, particularly inhibiting *Salmonella*, *Campylobacter*, and *E. coli* [19]. Nevertheless, the scalding process is accompanied by the accumulation of microorganisms in the water tanks. Specifically, there is a probability of detecting *Salmonella*, which survival increases with a decrease in a water temperature lower than 62 °C [20]. Thus, continuous water temperature monitoring enables minimizing the risk of porcine carcass contamination [21].

Proper evisceration is extremely important to ensure the sanitary and microbiological safety of meat. Microorganisms are constantly present in the gastrointestinal tract, internal organs, and their's lymphatic nodes. During evisceration, workers can accidentally cut the gastrointestinal tract, and meat and the production environment may be contaminated with the content of the digestive system. The studies show that the most contaminated sites when processing the porcine internal organs are the table for receiving and washing the stomach and the table for receiving and separating intestines [22].

¹ TR CU034/2013 Technical Regulations of the Customs Union "On the safety of meat and meat products" Retrieved from <http://docs.cntd.ru/document/499050564>. Accessed January 15, 2021. (In Russian)

Even healthy animals can be potential carriers of pathogens and opportunistic pathogens. The permeability of the intestinal walls changes as a result of stress during animal slaughter. Microflora of the gastrointestinal tract may penetrate other organs and tissues; therefore, its removal should be done as quickly as possible after slaughtering [19]. When collecting and processing intestinal raw materials, it is necessary to comply with strict sanitary and hygienic requirements, including those for washing and disinfection of production facilities [23].

The investigations indicate that different parts of porcine carcasses (ham, back, jowl and belly) after slaughter and their processing do not significantly differ in the microbiome composition. However, important differences in the carcass microflora were found when comparing carcasses from different slaughterhouses [24].

The effect of seasonality on the microbiological carcass status is ambiguous. On the one hand, it is believed that the highest risk of bovine carcass contamination with gut bacteria occurs in the summer and autumn [26]. Other studies showed that seasonality did not significantly influence microbiological indices of bovine, porcine, and ovine carcasses [27].

Various microorganisms are present on the wall and floor surfaces in production facilities, on the surfaces of equipment and utensils used in slaughterhouses. Among them, the most frequently detected are *Enterobacteriaceae*, *Proteus* spp., *Enterococcus* spp., *Staphylococcus* spp., and pathogens *Salmonella*, *Campylobacter*, *Yersinia enterocolitica* [28].

During the working shift, the hygienic condition of the floor and walls in a slaughterhouse significantly deteriorates. Analysis of swab samples shows that the total microbial counts exceeded the sanitary norm (1×10^3 CFU/cm²) by five times after 3 hours of slaughterhouse work, while by the end of the working shift (after 9 hours), they were $(1.6 \pm 0.23) \times 10^6$ and $(8.2 \pm 1.1) \times 10^5$ CFU/cm² on the floor and walls, respectively [22].

Cold chambers for chilled meat storage

Cold processing is an integral part of meat production. Hot meat chilling and creating the continuous cold chain at all following stages of the technological process are necessary to achieve stable quality and safety of meat products [29].

After slaughter and processing, hot carcasses should be immediately chilled. Exposure to low temperatures facilitates a decrease in spoilage microorganisms' growth rate and reduces the risks of pathogen growth [29].

Microflora of meat entering cold chambers for storage is diverse in composition and is usually represented by mesophiles, thermophiles, and psychrophiles [6].

During meat chilling, mainly psychrophilic and psychrotrophic microorganisms, such as gram-negative *Pseudomonas* and *Vibrionaceae*, gram-positive *Lactobacillus*, *Microbacterium*, *Arthrobacter*, microscopic fungi, yeasts, and coccal forms of microorganisms grow [6, 30].

The higher the initial meat contamination, the higher was the probability of fast multiplication of psychrophilic and psychrotrophic microorganisms during chilling [31, 32]. The presence of moisture on the meat surface can be favorable for microbial growth [33].

Pathogenic microorganisms can retain viability at low temperatures. In particular, *E. coli*, *Campylobacter* spp., *Clostridium perfringens*, *L. monocytogenes*, *Yersinia enterocolitica* were found in meat [34,35].

Intermittent spray-chilling of bovine carcasses using 1% acetic acid or 1% lactic acid slowed down microbial growth [36].

The study of the microbial species composition on the surfaces in the cooling chambers showed the presence of bacteria *B.subtilis*, *B.mesentericus*, *Pseudomonas* spp., *Sarcina flava*, yeasts *Rhodotorula*, mycelial fungi *Penicillium*, *Alternaria*, *Mucor*, *Aspergillus*, *Chryso sporium*, *Tamnidium*, *Cladosporium*. The highest numbers of microorganisms that survived after disinfection in the cooling chambers were observed on the surfaces of shelves, tables, and boxes for by-product storage [31].

The high level of the sanitary status of cooling chambers and adherence to storage conditions (temperature, relative humidity, airflow rate) facilitates reducing microbial meat contamination by one order of magnitude and ensures long-term storage of meat raw materials [31].

Meat cutting, boning, and trimming

High hygienic requirements are imposed on meat intended for processing as it is one of the contamination sources for production facilities and final products [37]. Microbial counts and species composition in cutting, boning, and trimming facilities are dependable on the air temperature and humidity. EAEU legislation establishes the requirement for the air temperature of not higher than 12 °C (TR CU034/2013²). However, this temperature is too high to effectively limit meat's microbial growth as its microflora is often represented by psychrophilic pathogenic microorganisms such as *Pseudomonas* spp., *L. monocytogenes*, *Yersinia enterocolitica* [38].

During carcass cutting, meat boning, and trimming, microbial counts increase due to many manual operations. Total microbial counts in meat can increase by 100 times and more compared to the initial values. During meat processing, cross-contamination can occur through cutting knives and contaminated working surfaces [39]. Sources of microbial contamination also include workers' hands and clothes, tools, utensils, the air in the production facilities, and so on [40,41]. As a result of carcass cutting and during the separation of bone-in and boneless cuts, microorganisms are transferred from the carcass and cut surfaces to the inner parts of muscle tissue. The area of meat contact with working surfaces and air increases, which, correspon-

² TR CU034/2013 Technical Regulations of the Customs Union "On the safety of meat and meat products" Retrieved from <http://docs.cntd.ru/document/499050564>. Accessed January 15, 2021. (In Russian)

dently, leads to deterioration of microbiological meat indices [42].

Microbiological examination of the wall, floor, and ceiling surfaces and the air of the production facilities in meat processing plants revealed that their sanitary and hygienic condition worsened as they were contaminated with microorganisms. For example, total microbial counts varied from 10^3 to 10^5 CFU/cm². Pathogens and opportunistic pathogens were present in the air, on the wall, floor, and ceiling surfaces, and the surfaces of the technological equipment and ventilation ducts (Lukin, A.A., Golubtsova, Yu.V.³).

Many authors emphasize the necessity to give particular attention to meat safety assurance and control of dangerous cold-tolerant microorganisms, including *Salmonella*, *E. coli*, *Staphylococcus*, *Pseudomonas*, *Listeria* [43,44,45].

Meat product manufacture

Meat product manufacture begins from the stage of preliminary meat processing (mincing, curing) and ends at the stage of its packaging. Fine meat grinding and minced meat preparation promote significant microbial growth. Microorganisms are distributed throughout minced meat, which is a favorable environment for their growth [46].

Microorganisms enter sausage meat from different sources: with meat, non-meat ingredients, especially non-treated spices, from air, workers' hands, and utensils [46,47].

When using spices and other recipe ingredients, particular attention is given to the incoming control of their compliance with the microbiological parameters established by regulations, for example, TR CU029/2012⁴ for the EAEU states.

Several studies have shown that spices contain high levels of aerobic microorganisms and pathogenic, spore-forming bacteria, yeasts, and molds [48,49]. In particular, increased numbers of *B. cereus* were found in the samples of ground black pepper. Spices are often contaminated with molds *Aspergillus* and *Penicillium* spp. [49]. There are also reports about many cases when *Salmonella* spp. and *Escherichia coli* were detected in spices and herbs such as basil, coriander, black pepper, and peppermint [50]. Dried vegetables and spices are also sources of lactic acid bacteria (LAB), some of which can cause food spoilage. The study results showed that lactic acid bacteria were found in ingredients in 65% of cases. Their content in dried onions and garlic powder was at the highest level. Among revealed LABs, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, and *W. confusa* were associated with food spoilage [51].

³ Lukin, A.A., Golubtsova, Yu.V. (2018, 14–16 May). *Research of the sanitary and hygienic well-being of raw materials, the surface of walls, floors, ceilings, air-gas space at a meat processing industry enterprise*. Innovations in food biotechnology. Proceedings of the International Symposium. Kemerovo, Russia (In Russian)

⁴ TR CU029/2012 Technical Regulations of the Customs Union "Safety Requirements for Food Additives, Flavorings and Technological Aids" Retrieved from <https://docs.cntd.ru/document/902359401>. Accessed May 7, 2021. (In Russian)

The hygienic properties of sausage casings also affect minced meat microflora. *B. halophilum*, various micrococci, *Sarcina*, aerobic bacilli, actinomycetes, molds, and other halophilic and salt-tolerant microorganisms have been found in natural casings preserved by the wet or dry salting methods [52]. Artificial casings are usually the most hygienic [53].

After minced meat stuffing, any additional external contamination of sausages can occur only upon cutting and packaging the finished product. The primary sources of microbiological contamination are workers' hands and equipment [54].

Risk assessment of finished product contamination with pathogens and spoilage microorganisms during production is essential in monitoring meat processing plants [55].

Among various microbial species, *L. monocytogenes* is the most dangerous one. The available data on *L. monocytogenes* in meat products indicate the different frequency and level of its detection [56, 57]. Analyses of frozen and chilled meat from various manufacturers, semi-finished meat products (lumpy and chopped), raw smoked and dry-cured sausages at different stages of aging, swabs from technological equipment and utensils showed high levels of *Listeria* detection [56]. Particular attention should be given to the cleaning and disinfection of equipment and surfaces in meat processing plants to prevent cross-contamination with *L. monocytogenes* [57].

The correlation between the frequency of *Listeria* spp. and *L. monocytogenes* detection in swabs taken in the production facilities was established, suggesting the possibility to use the presence of *Listeria* spp. as an indicator of the pathogen, reducing, thereby, time for analysis [58].

Sanitary and hygienic conditions of the air in industrial premises

Many studies are devoted to the microbiological assessment of the air in slaughterhouses [59,60]. Air contamination showed a decreasing trend being the lowest in the chiller compared to the early stages of the slaughter process [59].

Some microorganisms such as *E. coli*, *Salmonella*, and *L. monocytogenes* can survive and circulate in the air of production facilities contaminating the meat. The transmission of microorganisms through the air is influenced by:

- employees;
- moving parts (mechanisms);
- raw meat,
- insufficient separation between each zone;
- open drain ducts;
- plant structures,
- poor sanitary and hygienic conditions of production [60,61].

Employees have a significant impact on microbial air pollution. It was found that a slowly gesturing person can generate about 500,000 particles min⁻¹, and a rapidly ges-

turing person can produce up to 5,000,000 particles min^{-1} . During an 8-hour working shift, employees potentially can contaminate 8,000 l of air [61].

Sanitary treatment of industrial premises

In meat industry plants, sanitization is conducted to remove residues of raw materials, contaminants, including microorganisms, from working surfaces, including technological equipment and utensils, and other objects (floor, walls) [62].

Disinfection of wall and floor surfaces is critical in all production facilities, including those where animals are slaughtered. Examination of surfaces in the stunning and bleeding area was carried out before and after using the 1% disinfection solution containing surfactants, organic acids, and inorganic buffers [63]. The obtained results showed a possibility to reduce total bacterial counts, coliforms, and molds. It is believed that an increase in the hygienic level in slaughterhouse facilities will help meat industry professionals to establish proper sanitary procedures to prevent or reduce microbiological contamination of meat and meat products [63].

When choosing disinfectants, it is necessary to assess their effectiveness, quality, antimicrobial properties, toxicity, hazard class, corrosive activity, usability, ease of use, and economic feasibility [64].

The following factors affect the effectiveness of disinfection:

- physical and chemical properties of a disinfectant — the ability to inactivate bacteria, concentration, water-solubility, temperature, pH, etc.;
- biological resistance of microorganisms to various disinfectants;
- features of processed objects — the quality of materials, design features, the degree of pollution with organic substances;
- level of microbial contamination of fomites;
- disinfection treatment methodology — large-drop or aerosol irritation, wiping or immersion in a solution;
- exposure time [65].

Non-compliance with an established sanitization schedule, usage of ineffective disinfectants, or long-term application of the same disinfectant can lead to a decreased effectiveness of disinfection [66]. Microflora circulating in the food production environment may become resistant to disinfectants. Antimicrobial resistance is explained by the biofilm-forming ability of microorganisms [67]. The initial stage of biofilm formation is the attachment of microorganisms to the surface of production equipment and utensils covered with organic pollutants. This process is intensified until attachment becomes irreversible and strong [68].

Microflora protected by a biofilm becomes more resistant to disinfectants. Biofilm removal is a rather tricky task [69].

Timely preventive measures taken against molds are urgent for any food industry, including meat processing plants. Molds freely circulating in production facilities for fermented meat products can cause incredibly huge losses [70].

The choice of the most effective antifungal disinfectants and determination of adequate concentrations are of critical importance [71].

The following preparations at the indicated concentrations were used as liquid disinfectants: benzalkonium chloride (5%), biguanide (5%), peracetic acid (3%); quaternary ammonium (5%); sodium hypochlorite (0.2%).

The study [71] showed that the antifungal activity of peracetic acid was highest compared to the other tested chemical disinfectants. It was noted that some fungal strains, for example, *A. westerdijkiae* and *P. polonicum*, showed increased resistance to all disinfectants at the above concentrations.

Benzalkonium chloride and quaternary ammonium salts showed similar antifungal activity against the studied fungal strains. Sodium hypochlorite and biguanide at concentrations mentioned above had the lowest antifungal activity against molds in the production of dry-cured meat products [71].

It should be stressed out that biofilm formation by filamentous fungi must be considered in the selection of production environment and technological equipment sanitization programs. Several studies demonstrated that *Aspergillus* (*A. nigri* and *A. flavi*), *Penicillium*, *Cladosporium*, and *Alternaria* molds could form biofilms in the aquatic environment and on different abiotic surfaces [72].

Biofilm formation by *Candida* spp. remains to be the most discussed issue. The presence of the extracellular matrix (biofilm) that protects fungal cells against disinfectants may become an additional problem in the meat industry [73]. The simplest solution to this problem is to prevent biofilm formation through scheduled sanitization and strict adherence to preventive measures that minimize the probability of biofilm formation [74].

Conclusion

Nowadays, a wide range of research is devoted to hygiene and sanitation issues in food production facilities. Finished product safety is a result of adherence to many requirements at different production stages. Meat and meat product manufacture is associated with the highest risks. In the past few years, many studies have shown that prudent sanitary and hygienic measures are necessary at all stages of meat product manufacture. The formation of microflora in the production facilities begins from the stage of animal slaughter. Multiple factors take part in the development of the microbial community at each production stage. The recent studies demonstrated that sanitization of workshops must be planned considering the possible biofilm growth on the surfaces of objects in the production environment.

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