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EXPLORATION OF THE POTENTIAL RESERVOIRS OF *PSEUDOMONAS* SPP. BACTERIA AT MEAT PROCESSING FACTORIES AND POULTRY FARMS

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Abstract
One of the microorganisms that cause spoilage of meat during its storage is the bacteria Pseudomonas. To prevent contamination of the finished products with these bacteria, it is important to find the places at the enterprise where they aggregate. Within the framework of this study, the objects and premises of the production facilities at meat processing factories and poultry farms were explored to detect their contamination with bacteria of Pseudomonas spp. The potential reservoirs of those bacteria were defined at these plants. In addition, the species diversity of Pseudomonas was established at the production facilities environment at the enterprises. 27 production facilities environments (structures, equipment, package containers) were examined for the presence of bacteria with the method of washings. The samples were examined to detect Pseudomonas bacteria, with their subsequent identification with the method of time-of-flight mass spectrometry MALDI-Tof-MS. 487 strains of bacteria of the genus Pseudomonas were isolated, which strains are represented by 47 species. As a result of the study it was found that all 27 production facilities were contaminated with various species of Pseudomonas. From two to fourteen species of Pseudomonas bacteria were detected at all facilities. 12 facilities of the enterprise for slaughter and processing of broiler chickens were contaminated with Pseudomonas gessardii. Pseudomonas bacteria spp. (identification is traced down only to its genus) were found at 10 objects. Pseudomonas tolaasii and Pseudomonas brenneri were found at 9 and 8 objects, respectively. The surfaces of 6 objects demonstrated contamination with Pseudomonas chlororaphis ssp chlororaphis and Pseudomonas koreensis. Other Pseudomonas species were found at 1–5 sites. Pseudomonas fluorescens were detected at 8 pork processing plant sites, Pseudomonas gessardii were found at 5 sites. 4 sites were contaminated with Pseudomonas chlororaphis ssp. chlororaphis and Pseudomonas koreensis, 3 objects contained Pseudomonas tolaasii, Pseudomonas spp., Pseudomonas rhodesiae, Pseudomonas libanensis and Pseudomonas extremorientalis. The remaining species of Pseudomonas were found at one or two sites in the territory of the pork processing plant. It was found that all production environment sites, regardless of their distance from the raw materials and the finished products, were contaminated with Pseudomonas bacteria. At the same time, the sites that had no contact with the food products showed wider diversity of Pseudomonas species than in the places where the contact took place. Thus, all the explored objects of the production environment at the pork processing enterprises and the facilities for slaughter and processing of broiler chickens are the potential reservoirs of Pseudomonas bacteria.

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Introduction

Modern technologies of food production and sanitization have changed the distribution and spatial arrangement of bacteria within the enterprises, and have led to circulation and contamination of the objects with microorganisms in the production sites. This is particularly acute issue for the production environment objects and facilities. Some are very difficult to sanitize, and due to it the organic residues and moisture accumulate and build up there for a long time. Such objects serve as reservoirs of various microorganisms, and upon contact with them, food products are contaminated [1]. Microorganisms at processing plant facilities can be either accidental contaminants or can be those microorganisms that have survived after sanitation due to their resistance to various factors.

Scientists around the world keep studying reservoirs of bacterial contamination in the food processing plants.

It is necessary to take into account that the objects of the meat processing flow line (equipment, auxiliary inventory, structures, etc.), which are made mainly of stainless steel (pipelines, hooks, knives), plastic (teflon), (e. g. conveyor belts) and polymers (polymer self-leveling floors) can be colonized by microorganisms. Microorganisms contaminate solid objects or the areas inaccessible for cleaning, both inside and outside of the equipment, like cracks, slots, holes and hollow parts, gaskets, unpolished or worn mate-

rials. They are the potential hideouts for the microorganisms and the reservoirs of resistant bacteria [2,3].

When studying the surface of the objects of the production environment in a meat processing plant, a high level of microorganisms of the phyla *Proteobacteria, Bacteroidetes, Firmicutes* and *Actinobacteria* was observed. It is necessary to clarify that the phyla *Proteobacteria, Bacteroidetes* and *Firmicutes* include cold-resistant microorganisms that cause food spoilage. Despite various hygiene practices, the microbiota in standard rooms and in the premises which require high level of hygiene, had no significant differences [4].

166 resistant mesophilic and psychrotrophic strains of *Pseudomonas* spp. were isolated from the surfaces of production environment objects (sinks, drains, walls, tables, equipment and floors) in a goat and sheep slaughter and processing premises (Jaén, Spain), in the cattle reception areas, slaughter premises, and all cold storage and cutting rooms [5].

The most contaminated objects in the production environment are the walls and floors. Floors are one of the main sources of microbial contamination, since their microbiota is transferred to various areas of the enterprise with the shoes of constantly moving employees, with the wheels of carts, and also during sanitization by high-pressure washing equipment. This method of floors cleaning spreads microorganisms because it sprays them into the air of the room in the form of aerosols [6].

Wastewater traps in the industrial premises is another hideout of microorganisms, in particular *Pseudomonas* spp. and *Aeromonas* spp., as these traps provide a favorable environment for microorganisms growth [7].

Another reservoir of microorganisms are the water supply hoses used for a long time at the enterprise (e. g. in meat processing ones). They contained biofilms with a complex and unique microbial community, represented by bacteria of the genus *Pseudomonas, Microbacterium* and *Psychrobacter.* The microbial composition of the biofilms in the hoses collected from one room was diverse and different. Opportunistic pathogens *Neochlamydia, Legionella* and *Pseudomonas* were detected. In two hoses *Pseudomonas* and *Microbacterium* were detected, and in water from them they found bacteria of the genus *Pseudomonas.* Very often the microbial composition of water (n = 5) correlated with the microbial composition of the water hoses internal surfaces. It is very important at food enterprises to ensure the supply of high-quality and safe water, since it is used at many stages of processing and for sanitization of food production facilities [8,9,10].

The constantly replenishable depots of microorganisms at the meat processing plants are the dirty skins of animals delivered for slaughter. Visually dirty skins and hides provided a large number of indicator microorganisms (total microbial count and *Enterobacteriaceae*) on the carcasses. Skins, in particular the cattle skins, are the main source of microbial contamination not only for the carcasses (during their skinning), but also of every object located within the animal slaughter line. It has been established that 1 cm^2 of cattle skin can contain up to 11 log_{10} CFU of aerobic bacteria [2].

Microbiome mapping at pigs processing premises has shown that bacteria like *Pseudomonas, Acinetobacter, Psychrobacter, Stenotrophomonas, Brevundimonas, Acidovorax* and *Microbacterium* dominate among the others. This microbiota is common to all studied objects and indoor structures of the premises [11].

Hundreds of various species may be found at a one food enterprise, but only a few taxa of resident microorganisms dominate. The taxonomic profile of various objects in the production environment was studied for over 18 months long. Bacteria of the genus *Pseudomonas,* which were the most numerous, gradually became secondary, although they still remained among the main microorganisms found in drain waters in the traps, on the equipment and on the tables. Bacteria of the genus *Pseudomonas* are of interest due to their acknowledged role in food spoilage processes. They were often detected after sanitization on the surfaces of the objects. Moreover, their resistance is probably explained by their low requirements for growth factors, their ability to grow at low positive temperatures and to form biofilms, as well as their ability to resist to biocides. Gram-negative bacteria, especially *Pseudomonas* spp., followed by *Enterobacteriaceae* and *Acinetobacter* spp., dominate over the surfaces of many objects in the production premises of food processing enterprises [12].

As for the pork processing, it is critical to understand on the example of slaughtering and processing poultry how the microflora of the final product is affected along the various stages of the production process. High microbial load is defined at a plant at the moment when the poultry is slaughtered. Microorganisms are distributed throughout the plant through various objects, staff and the air flows. All this factors, require continuous sanitization of equipment and the air environment. However, the producers consider speeding up the poultry processing line as a main priority, because it's necessary to satisfy consumers' increasing demand; this reduces the frequency of sanitizations at the plant. Broiler chicken meat can be contaminated with more than 280 microorganisms geni [13,14,15].

The next object that can be a reservoir of a large number of microorganisms is scalding vats water. On the surface of carcasses about 20 phyla of microorganisms still survive even after carcass scalding in hot water, respectively these same microorganisms remain in the water. About 60–70% of all types of microorganisms found in water were the phyla *Firmicutes, Proteobacteria* and *Actinobacteria.* High microbial contamination of scalding vats water was observed at the beginning of the working day. After several hours of water using, phylum-specific changes occurred. The number of microorganisms of the phylum *Proteobacteria* remained stable (it did not change), the number of microorganisms of the phylum *Bacteroidetes* decreased, the number of microorganisms of the phylum *Firmicutes,*

on the contrary, increased. It should be remarked that microorganisms of the phylum *Proteobacteria,* i. e. *Pseudomonas* and *Acinetobacter,* provided particularly negative effect on the poultry meat quality [16].

The hard rubber fingers of the carcass feather removal equipment serve as a depot for physical contaminants (faeces, dust and dirt) and the microorganisms they spread around. Feather removal can be considered one of the two main ways of poultry carcasses contamination, when bacteria from one poultry carcass are transferred to another one. The second way of contamination is poultry carcass evisceration. All objects of the production environment involved in these operations can be considered reservoirs of various microorganisms [16,17].

When carcasses are air-cooled, condensation drops are formed on the walls, which flows down onto the floor bringing along with them the microbial contaminants, in particular psychrophilic lactic acid bacteria, *Pseudomonas* and pathogenic *Listeria.* spp. During sanitization of the floor, they are spread around the room in aerosols. At the stage of cutting up the poultry carcass, employees and equipment serve as vectors of cross-contamination for these microorganisms [14,18].

The most common bacteria found on poultry meat while its storage under aerobic conditions are proteolytic species of *Pseudomonas* spp., which account for up to 58.5% of all isolated geni of microorganisms. More than 111 species of *Pseudomonas* have been identified on poultry meat [19,20].

Bacteria of the genus *Pseudomonas* are considered as the most heterogeneous group of gram-negative bacteria, they are represented by aerobic, motile, catalase-positive and non-spore-forming rods. These bacteria are all-pervasive and are found in soil, fresh water, humans, on the surface of the plants and animals, and in the animal and plant products. Bacteria of the genus *Pseudomonas* are opportunistic microorganisms. They cause various infectious diseases in humans and animals [21,22,23,24,25], as well as plants [26]. In addition, these bacteria cause spoilage of food products, including chilled ones [27,28]. They cause taste deterioration, rotting, rancid odor, liquefaction of the food product and the mucous deposits formation, for example on the cheese surface. They give an unpleasant taste to protein products with high water activity (meat, dairy products) and fish products due to the volatile compounds formation and amino acids breakdown; change the color of minimally processed vegetables due to their pectinolytic activity, and also start lipolysis and proteolysis of processed milk due to heat-resistant enzymes. *Pseudomonas fragi, Pseudomonas putida* and *Pseudomonas fluorescens* are particularly active in causing spoilage.

Based on the analysis of scientific publications, it was established that there is interest to studying the main sources of microbial contamination of food products at the food processing plants. The main foci of these researches are the study of a wide range of microorganisms, while only few publications are devoted to studying of a particular genus that causes spoilage and reduces the shelf life of the food products.

The purpose of this study is to explore the objects in the production environment of meat processing plants and poultry farms for their contamination with bacteria *Pseudomonas* spp., and to detect the potential reservoirs of these bacteria in these production facilities.

Objects and methods

Production environment objects of a pork processing plant

Wall in the receiving bin for pig half-carcasses; Wall of the raw material workshop (opposite the conveyor with half-carcasses); Conveyor (metal) for cutting pig half-carcasses next to the band saw; Band saw body (raw material workshop); Saw blade (metal) of the band saw in the raw material workshop; Trap/conveyor (metal) at the point of sawing half-carcasses into the cuts; Conveyor (polymer material) for clean containers delivery; Belt (polymer material) of the finished product feeding line; Manual pallet forklift (metal) for transporting pallets; Wall (metal) of the elevator in the container washing room for lifting dirty containers.

Objects of the production environment at the enterprise for slaughter and processing of broiler chickens (BC)

The outer surface (stainless steel) of the water cooling vat for chicken carcasses; The underside of the steps of the cooling vat trap (stainless steel); The ceiling above the cooling vat for BC carcasses, painted with paint; Roller (teflon) of the conveyor for moving BC carcasses during their cooling; Chain (metal) of the conveyor for hanging BC carcasses after water cooling; The frame (stainless steel) of the machine for weighing and re-hanging BC carcasses; Fixed parts (stainless steel) of the machine for deboning BC thighs, contaminated with meat juice; The frame of the machine for deboning BC thighs without visible contamination with meat juice; Chain (metal) of the machine for BC carcasses evisceration; Belt (polymer material) of the equipment for BC meat packing; Container (plastic) for collecting BC carcasses; Wall (tile) at a distance of 1.60 m from the floor; Table (stainless steel) for shaping and arranging BC carcasses before packaging; Two-tier trolley (stainless steel); Wheel (polymer material) of a floor trolley for transporting raw materials; Trap (stainless steel) in the BC carcasses evisceration workshop; Trap (plastic) in BC carcasses evisceration workshop.

Bacterial strains *Pseudomonas* spp. isolated from the objects in the production environment.

Swabs sampling

The swabs were sampled during the work process. In order to study objects for the presence of bacteria of genus *Pseudomonas,* the swabs were taken from 100 cm^2 area of the surface. In case of uneven surfaces, the swabs were collected without reference to the area. The swabs were sampled with sterile sponges (3M Dry-Sponge, USA), premoistened in 10 cm^3 of buffered peptone water. The spong-

es were transported to the lab study site in sterile packages at a temperature of 1 °C to 4 °C to prevent contamination.

Isolation and identification of strains

From a sponge bag aliquot of 100 µl of liquid volume was taken and distributed with sterile spatula onto the surface of CFC agar (cephalothin-sodium fucidin -citrimide agar) (Oxoid, UK) in a Petri dish. After 72 hours of culturing the crops at a temperature of 24 °C, colonies were selected for their species identification using the MALDI-Tof-MS time-of-flight mass spectrometry method on Autof MS1000 mass spectrometer (Autobio Diagnostics, China). For this purpose, the bacterial mass of colonies was applied onto a plate/target and dried at room temperature. Then, 1.2 µl of formic acid was applied to each cell with the dried bacterial mass for 10 min, dried up, 1.2 µl of the HCCA matrix (a-cyano-4-hydroxycinnamic acid, 99%) was applied onto dry mass and dried again. The MALDI target was placed in the device and the equipment for identification of microorganisms was launched using the FlexControl program (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 for the further study. The result was considered as reliable and was taken into consideration when the values accounted for 6.0–9.0 — at the genus level, 9.0–9.5 — at the species level.

Results and discussion

To establish the presence of bacteria of the genus *Pseudomonas* at enterprises processing meat of the slaughtered animals (pigs) and poultry, the objects of the production environment were selected both being in direct contact with raw materials and finished products, and quite remote from them. At the pork processing enterprise 10 objects were assessed, at the BC slaughtering and processing enterprise — 17 objects. Microorganisms isolated from these objects were identified, and for the further analysis only data on bacteria of the genus *Pseudomonas* were used. The fact of the presence of the sought microorganisms was established on the basis of detection of 487 strains of genus *Pseudomonas* bacteria. Each of them was identified up to the species, or to the genus if the species profile of the identified strain in the device database was absent.

The study established that the bacteria *Pseudomonas* spp. were present in a pork processing plant and a poultry slaughter and processing plant, and were represented by 47 species.

Researches of many scientists show that pseudomonads may be present at the enterprises for several reasons. First of all, it happens due to constantly replenishable sources of these bacteria, such as animal skins, bird feathers, water and biofilms on various surfaces (e. g. in hoses, in hard-to-reach places) [2,8,9,10]. In addition, there are resistant "industrial" strains of these microorganisms. The mechanism of microorganisms' transmission is the presence of contaminated objects and/or their sanitization with high-pressure equipment, when microorganisms are transferred via long distances due to aerosols. In addition, after sanitization, the surface of the equipment remains wet, and water is the main reservoir of *Pseudomonas* genus bacteria.

To determine the sources of which species of *Pseudomonas* spp. is some particular object, a list of species isolated from the surface of each object was drawn up. The results are presented below in the Tables 1 and 2.

Table 1. Species diversity of *Pseudomonas* at the production facilities of a pork processing plant

	Objects of research	*Pseudomonas* species	*Pseudomonas* species number
1	Wall in a receiving bin for pig half-carcasses	*Pseudomonas brenneri* *Pseudomonas fluorescens* *Pseudomonas fragi* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas mandelii* *Pseudomonas* spp.	n = 7
2	Wall of the raw materials workshop (opposite the conveyor with half carcasses)	*Pseudomonas cedrina* *Pseudomonas fluorescens* *Pseudomonas koreensis* *Pseudomonas rhodesiae* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 6
3	Conveyor for cutting pig half-carcasses next to a band saw	*Pseudomonas fluorescens* *Pseudomonas gessardii* *Pseudomonas graminis* *Pseudomonas rhodesiae*	n = 4
4	Band saw body (raw materials workshop)	*Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas proteolytica*	n = 3
5	Band saw blade (raw materials workshop)	*Pseudomonas azotoformans* *Pseudomonas chlororaphis* *Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas libanensis* *Pseudomonas putida*	n = 8
6	Trap/conveyor at the point of cutting half-carcasses into the cuts	*Pseudomonas brenneri* *Pseudomonas chlororaphis* *Pseudomonas koreensis* *Pseudomonas tolaasii*	n = 4
7	Conveyor for clean containers delivery	*Pseudomonas chlororaphis* *Pseudomonas fluorescens* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas taetrolens*	n = 5
8	Finished product feed line belt	*Pseudomonas chlororaphis* *Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas gessardii* *Pseudomonas grimontii* *Pseudomonas libanensis*	n = 6
9	Manual pallet forklift	*Pseudomonas fluorescens* *Pseudomonas fragi*	n = 2
10	Wall of the lift in the container washing room for lifting dirty containers	*Pseudomonas aeruginosa* *Pseudomonas gessardii* *Pseudomonas jinjuensis* *Pseudomonas koreensis* *Pseudomonas rhodesiae* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 7

Out of the 10 objects studied at the pork processing plant, only 5 had direct contact with raw meat and finished products. One of them was a band saw blade located in the raw materials workshop. It was found to contain the most types of bacteria *Pseudomonas* (n = 8). The saw blade may be a potential source of contamination, since it is not sanitized after each carcass cutting. On the body of the same band saw there were significantly fewer *Pseudomonas* species (n = 3). In similar studies by Japanese scientists, *Pseudomonas* spp. were also found in the swabs taken from the handle of a brisket saw, from the hooks and from the aprons of the staff in the boning workshop. At some sites they were found even after sanitization [29]. A conveyor for delivery of clean containers, which should be free of all types of contamination, was found to be contaminated with five species of *Pseudomonas*.

On objects that had no direct contact with raw materials, such as the wall of the storage bin, the wall of the elevator for lifting dirty containers to the container washing room, *Pseudomonas* (n = 7) was detected in lesser amounts, but not significantly lesser. Among the bacteria detected in the elevator, the bacteria pathogenic for humans were found — *Pseudomonas aeruginosa*. On the wall opposite the conveyor with pig half-carcasses, 6 species of *Pseudomonas* were found, including those that generate pigment when growing on meat. *Pseudomonas* bacteria were also found on the belt of the finished product feed line and on the conveyor for cutting up half-carcasses near the band saw and on the trap/conveyor of the point where half-carcasses are cut into the cuts.

Exploration at the pork processing plant found that all 10 production objects were contaminated with various species of *Pseudomonas* bacteria, which fact meant they were all sources of microbial contamination.

According to the research data presented in the work [30], the number of *Pseudomonas* spp. varies depending on the type of industrial activities of the enterprise. The samples collected from the dairy industry demonstrated the highest average count of *Pseudomonas* spp. among all other industries studied, followed by the samples taken from pork and poultry processing plants ($P < 0.05$).

We have obtained very interesting results of bacterial colonization by *Pseudomonas* spp. not only at pork processing plants, but also at the poultry processing ones, which proves the diversity and abundance of microorganisms in these industries.

The results of the study of the production environment objects at the enterprise for BC slaughter and processing are presented below in the Table 2.

To establish a deep fundamental understanding of bacterial relationships and changes in poultry meat and at production facilities (from breeding to distribution), it is necessary to have data on the microbiota along the entire poultry production chain.

Our findings can help in developing quality and safety management measures in poultry processing plants. It is

Table 2. *Pseudomonas* species diversity in the production environment at the enterprise for BC slaughter and processing

No	Objects of research	Types *Pseudomonas*	*Pseudomonas* species
	External surface (stainless steel) of the vat for water cooling of BC carcasses	*Pseudomonas brenneri* *Pseudomonas fluorescens* *Pseudomonas gessardii* *Pseudomonas graminis* *Pseudomonas* spp.	n = 5
	Underside of the stairs (stainless steel) of the cooling vat	*Pseudomonas lundensis* *Pseudomonas poae* *Pseudomonas proteolytica* *Pseudomonas* spp. *Pseudomonas trivialis*	n = 5
	Ceiling above the cooling vat of BC carcasses, painted with paint	*Pseudomonas chlororaphis ssp chlororaphis* *Pseudomonas lundensis*	n = 2
	Roller (teflon) of the conveyor for moving BC carcasses during their cooling	*Pseudomonas corrugata* *Pseudomonas gessardii* *Pseudomonas veronii*	n = 3
	Chain (metal) of the conveyor for hanging BC carcasses after water cooling	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas chlororaphis* *Pseudomonas gessardii* *Pseudomonas grimontii* *Pseudomonas koreensis* *Pseudomonas marginalis* *Pseudomonas proteolytica* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 10
	Frame (stainless steel) for BC carcasses weighing and re-hanging machine	*Pseudomonas corrugata* *Pseudomonas mendocina* *Pseudomonas putida* *Pseudomonas* spp.	n = 4
	Fixed parts (metal) of the BC thigh deboning machine contaminated with meat juice	*Pseudomonas brenneri* *Pseudomonas chlororaphis* *Pseudomonas gessardii* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas lundensis* *Pseudomonas putida* *Pseudomonas synxantha* *Pseudomonas syringae* *Pseudomonas tolaasii*	n = 10
	Frame (without visible contamination with meat juice) of the machine for BC thighs deboning	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas extremorientalis* *Pseudomonas flavescens* *Pseudomonas fragi* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas lundensis* *Pseudomonas putida* *Pseudomonas rhodesiae*	n = 10
	Chain (metal) of the machine for BC carcasses evisceration	*Pseudomonas chlororaphis* *Pseudomonas corrugata* *Pseudomonas extremorientalis* *Pseudomonas marginalis* *Pseudomonas* spp.	n = 5
	Tape (polymer material) for BC meat packaging equipment	*Pseudomonas brenneri* *Pseudomonas koreensis* *Pseudomonas gessardii* *Pseudomonas libanensis* *Pseudomonas mandelii* *Pseudomonas savastanoi ssp savastanoi* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 8

Table 2. End

No	Objects of research	Types *Pseudomonas*	*Pseudomonas* species
	Container (plastic) for collecting BC carcasses	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas fluorescens* *Pseudomonas fragi* *Pseudomonas gessardii* *Pseudomonas graminis* *Pseudomonas orientalis* *Pseudomonas synxantha* *Pseudomonas tolaasii*	n = 9
	Wall (tile) at a distance of 1.60 m from the floor	*Pseudomonas aeruginosa* *Pseudomonas brenneri* *Pseudomonas frederiksbergensis* *Pseudomonas fuscovaginae* *Pseudomonas gessardii* *Pseudomonas grimontii* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas mendocina* *Pseudomonas mosselii* *Pseudomonas proteolytica* *Pseudomonas putida* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 14
	Table (stainless steel) for shaping and arranging BC carcasses before packaging	*Pseudomonas azotoformans* *Pseudomonas brenneri* *Pseudomonas gessardii* *Pseudomonas mucidolens* *Pseudomonas putida*	n=5
	Two-tier trolley (stainless steel)	*Pseudomonas agarici* *Pseudomonas corrugata* *Pseudomonas gessardii* *Pseudomonas* spp. *Pseudomonas tolaasii*	n = 5
	Wheel (polymer material) of a floor trolley for raw materials transporting	*Pseudomonas brenneri* *Pseudomonas chlororaphis ssp aurantiaca* *Pseudomonas corrugata* *Pseudomonas extremorientalis* *Pseudomonas fluorescens* *Pseudomonas fragi* *Pseudomonas gessardii* *Pseudomonas koreensis* *Pseudomonas libanensis* *Pseudomonas proteolytica* *Pseudomonas* spp. *Pseudomonas stutzeri* *Pseudomonas tolaasii*	n = 13
	Trap (stainless steel) in the BC evisceration workshop	*Pseudomonas fragi* *Pseudomonas frederiksbergensis* *Pseudomonas gessardii* *Pseudomonas libanensis* *Pseudomonas lundensis* *Pseudomonas* spp. *Pseudomonas tolaasii* *Pseudomonas trivialis* *Pseudomonas veronii*	n = 9
	Trap (plastic) in the BC evisceration workshop	*Pseudomonas chlororaphis* *Pseudomonas corrugata* *Pseudomonas fulva* *Pseudomonas koreensis* *Pseudomonas tolaasii*	n = 5

important to prevent cross-contamination during slaughter and processing via management of the employees' hygiene and the production environment.

Pseudomonas bacteria (n = 14) was found in wall swabs collected at the evisceration site at a height of 1.6 m from the floor at BC slaughter and processing plant. This premise does not come into contact with products, but its microbial composition may serve as a source of *Pseudomonas* spp. spreading over the other objects through high-pressure washing. Among the 14 species of *Pseudomonas,* two species pathogenic to humans were found: *Pseudomonas aeruginosa* and *Pseudomonas mendocina* [31]. It is possible that this object is not sanitized regularly and efficiently. In the case of condensation on the wall, along with the drops 14 species of *Pseudomonas* spp. will move down to the floor, and when the floor is sanitized with high-pressure washing, they will move up in aerosols through the air and spread over equipment and finished products. *Pseudomonas* bacteria can also move along the floor on the wheels of a cart, both inside and outside of the premises.

Pseudomonas bacteria (n = 13), predominantly from the group of *Pseudomonas fluorescens,* were also found on the wheel of the floor trolley used to transport the raw materials.

Third place in species diversity of *Pseudomonas* spp. in the slaughterhouse and processing plant is occupied by the chain of the conveyor for BC carcasses hanging (after water cooling), the stationary parts of BC thigh deboning machine contaminated with meat juice, and the frame of BC thigh deboning machine. On their surfaces 10 species of *Pseudomonas* were found, of which two were common to all three sites: *Pseudomonas brenneri* and *Pseudomonas gessardii. Pseudomonas brenneri* features proteolytic activity and when growing on a protein matrix generates an unpleasant odor of aldehydes, ketones and esters. *Pseudomonas gessardii* conduct lipolytic and proteolytic activity [27,28]. In case of cross-contamination of poultry products containing large amounts of protein and fat with these *Pseudomonas* species, spoilage processes are possible.

An analysis of the production environment confirmed our assumption about the possibility of contamination of food products upon their contact with the industrial objects. Nine species of *Pseudomonas* were found in the container for collecting BC carcasses, most of which are the microorganisms that contribute to spoilage process. This means that collecting BC carcasses into this container will contaminate them.

Two species of *Pseudomonas* were also found in the swabs from the ceiling above the cooling vat, which vat was painted with paint. If condensation formed on it, there was a risk of contamination of the food products.

The remaining 10 objects in the production environment of the slaughterhouse and processing plant were also contaminated with various species *of Pseudomonas.* These objects can be considered as potential depots *of Pseudomonas* spp.

If to sanitize only the objects of production environment that contact with raw materials and finished products, then the objects that do not come into contact can become depots of various microorganisms.

By analyzing the frequency of various species *of Pseudomonas* occurrence, it was found that out of 487 strains collected from industrial environment objects, 28 species *of Pseudomonas* were detected in no more than 1.0% (n = 50) of each. Among them were strains of bacteria pathogenic to humans — *Pseudomonas aeruginosa* (0.62%, n = 3) and *Pseudomonas mendocina.*

The remaining 19 species *of Pseudomonas* showed more strains. For example, the share of *Pseudomonas mosselii, Pseudomonas synxantha, Pseudomonas azotoformans, Pseudomonas frederiksbergensis, Pseudomonas mandelii* and *Pseudomonas rhodesiae* accounted for 1.03–1.85% (n = 44) of the total number of strains (n = 487). *Pseudomonas lundensis, Pseudomonas putida, Pseudomonas extremorientalis* and *Pseudomonas koreensis* were found in quantity of 2.26–2.87% (n = 50) of strains. *Pseudomonas proteolytica, Pseudomonas fluorescens* and *Pseudomonas libanensis* showed slightly bigger quantity — 4.11–4.93% (n = 67) of strains. The species of *Pseudomonas fragi, Pseudomonas tolaasii* and *Pseudomonas chlororaphis* represented 5.13–5.54% (n = 78) of the strains.

Most of the isolated strains were represented by two species: *Pseudomonas gessardii* (12.53%, n = 63) and *Pseudomonas brenneri* (20.53%, n = 100). However, for 7.19% (n = 35) of the strains, it was not possible to determine the species affiliation, since the device's software reliably identified them only down to the genus *Pseudomonas* spp.

All 47 *Pseudomonas species* were distributed across facilities, thus making it possible to determine which facilities in the enterprise are the reservoirs of certain *Pseudomonas species.*

According to foreign authors, bacteria of *Pseudomonadaceae* family, consisting of the genus *Pseudomonas* dominated over the place at the slaughter stage by an average of 14.71%, at the processing stage — by 29.05%, and at the sales stage — by 30.54%. They were detected in 27.23% of the floor swabs samples analyzed. The relative abundance *of Pseudomonas* remained high at the stage of poultry rearing, slaughter, processing and sale stages. Some *Pseudomonas* species, such as *P. fragi,* now are widely recognized as the dominant species associated with spoilage of meat products and are found in BC microbiota [30].

For assessing the frequency of particular species detection on the objects, the data on *Pseudomonas* species from the Tables 1 and 2 were presented in the form of diagrams. It was not possible to find a similar assessment of the data in the studies of the other researchers.

Figures 1 and 2 show the diagrams *Pseudomonas* species distribution over the objects of the production environment at enterprises.

In result of study of ten objects in the production environments of pork processing plant, it was found that eight of them (80.0%) were contaminated with *Pseudomonas fluorescens,* five (50.0%) — with *Pseudomonas gessardii.* Four objects (40.0%) were infected with *Pseudomonas chlororaphis* ssp. *chlororaphis* and *Pseudomonas koreensis.*

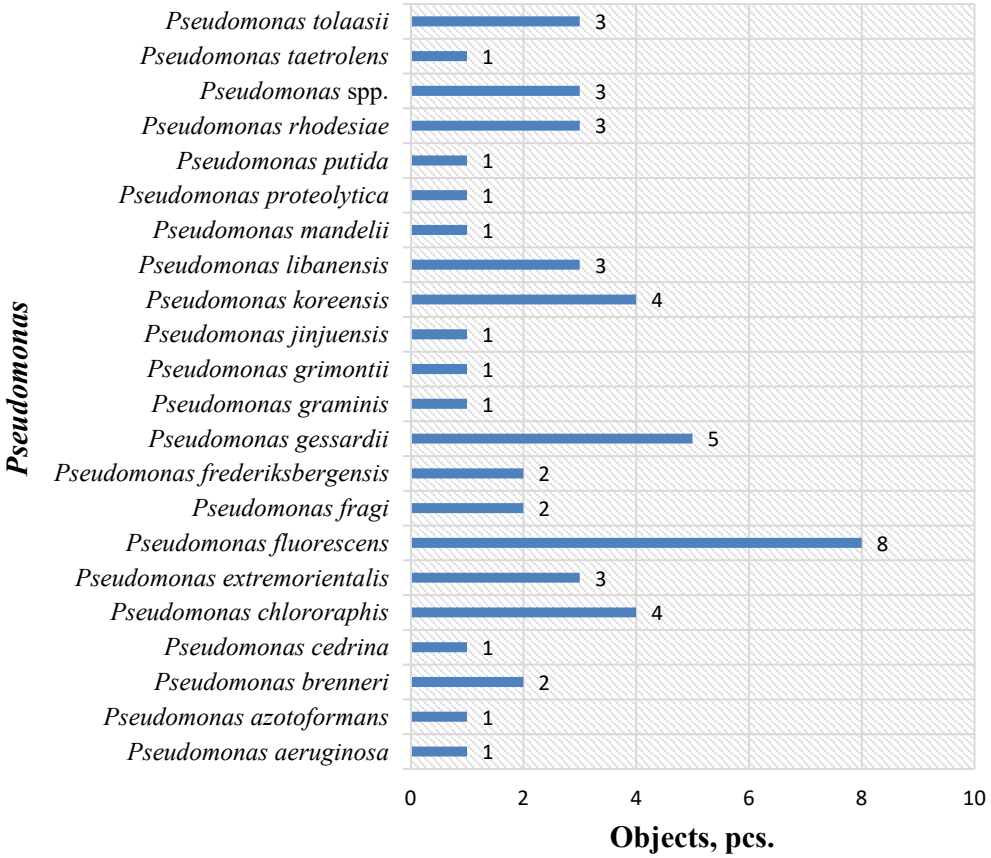

Figure 1. Distribution of various *Pseudomonas* species (n = 22) among the production objects of a pork processing plant

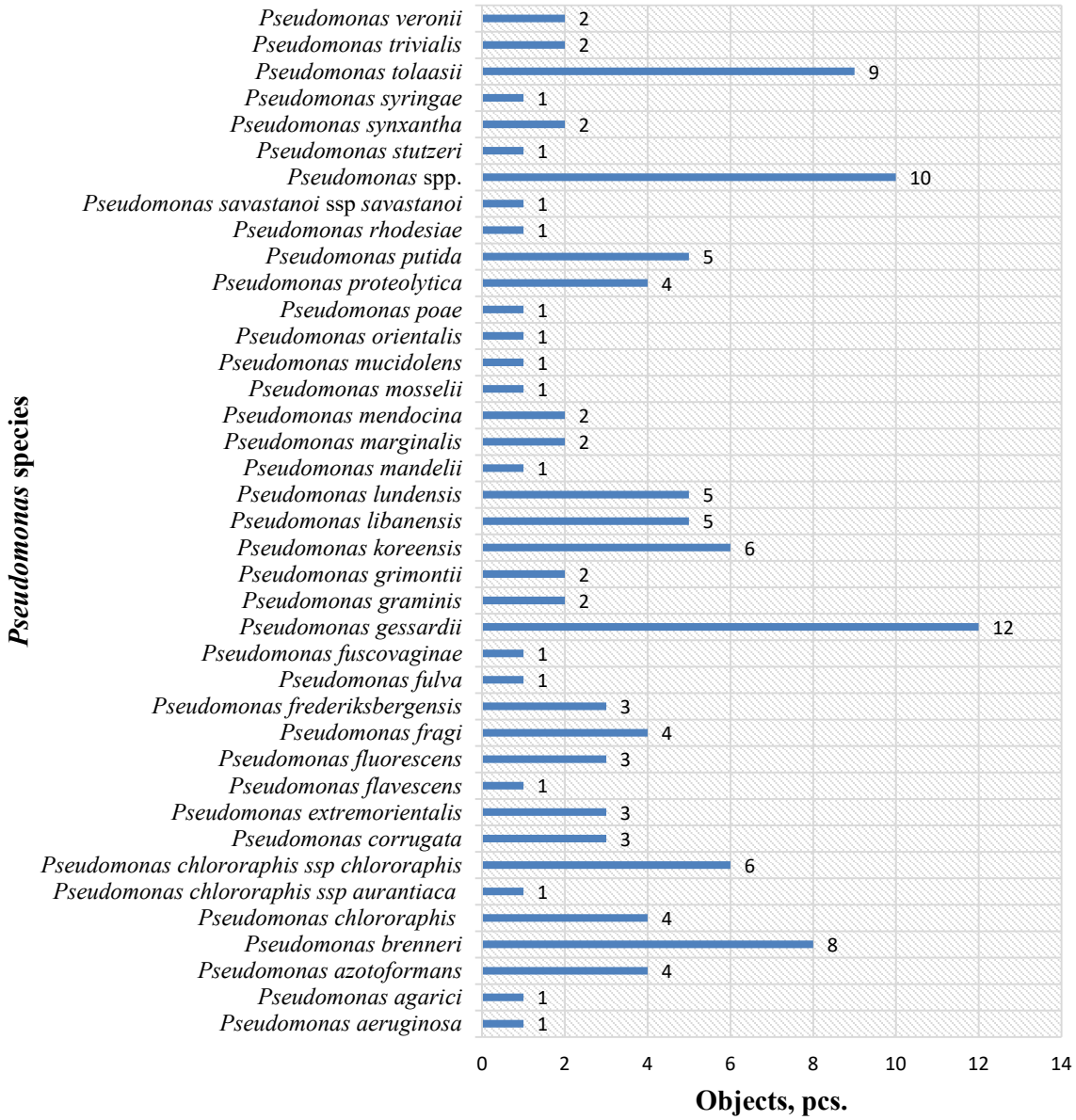

Figure 2. Distribution of various *Pseudomonas* species (n = 39) among the production objects of BC slaughtering and processing plant

Three objects (30.0%) contained *Pseudomonas tolaasii*, *Pseudomonas* spp., *Pseudomonas rhodesiae*, *Pseudomonas libanensis* and *Pseudomonas extremorientalis*. The rest of *Pseudomonas* species were detected at one or two sites, which made up 10.0 to 20.0% of their occurrence. At this pork processing facility *Pseudomonas fluorescens, Pseudomonas gessardii, Pseudomonas chlororaphis* ssp. *chlororaphis* and *Pseudomonas koreensis* were the most predominant species.

Out of 17 objects in the production objects of the enterprise for BC slaughtering and processing, 12 of them (70.6%) were contaminated with *Pseudomonas gessardii*. On the surface of 10 (58.8%) objects some strains could be identified only down to the genus *Pseudomonas*. *Pseudomonas tolaasii* were detected on 9 (52.9%) objects, and *Pseudomonas brenneri* were found on 8 (47.1%) objects. The surface of 6 (35.3%) objects contained *Pseudomonas chlororaphis* ssp *chlororaphis* and *Pseudomonas koreensis*. Other *Pseudomonas* species were detected at 1–5 (5.9% — 29.4%) sites.

At this slaughterhouse and processing plant most of the objects were contaminated with *Pseudomonas gessardii, Pseudomonas* spp., *Pseudomonas tolaasii* and *Pseudomonas brenneri*.

Conclusion

The results of the study showed that all 27 objects of the production environment at meat processing and poultry enterprises were contaminated with 47 species of bacteria of *Pseudomonas* genus. Many of these bacteria species affect the organoleptic properties of food products, even if the food products are stored in full compliance with storage conditions, and in this case meat products become a source of bacteria of *Pseudomonas* genus. The obtained data showed that *Pseudomonas gessardii* is the common *Pseudomonas* species among the industrials objects of the pork processing plant and BC slaughtering and processing enterprise. In addition to this microorganism, the pork processing plant features the dominating presence of *Pseudomonas fluorescens, Pseudomonas chlororaphis* ssp. *chlororaphis* and

Pseudomonas koreensis; at the slaughtering and processing plant *Pseudomonas* spp., *Pseudomonas tolaasii* and *Pseudomonas brenneri* dominate. It should be noted that all objects of the production workshops, regardless of their distance from raw materials and the finished products, are contaminated with *Pseudomonas* bacteria. At the same time on the surface of the objects that did not come into direct contact with the products the diversity of *Pseudomonas* species was higher than at those objects which had direct contact. The obtained data prove the necessity to revise the sanitization program in terms of the list of objects for sanitization, and to include the microbiological parameter "*Pseudomonas* spp. bacteria" into the check list of the production control program for the finished products monitoring.

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