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TESTING OF METHODS FOR DETECTING *SALMONELLA* IN THE AIR OF POULTRY PROCESSING PLANTS

Yulia K. Yushina, Dagmara S. Bataeva*, Anastasia A. Semenova, Maria A. Grudistova, Elena V. Zaiko, Anzhelica A. Makhova
V. M. Gorbatov Federal Research Center for Food Systems, Moscow, Russia

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Abstract
The aim of the study is to compare the effectiveness of microbiological and PCR methods for detecting Salmonella in the air at various technological sites in four poultry processing plants and in one poultry farming enterprise. The objects of the study were air samples collected on two nutrient agars: non-selective PCA agar and selective XLD agar. Microbiological and PCR methods were used. Air samples collected on PCA agar were cultured in the BPW (enrichment stage). The culture liquid obtained in this way was used in the isolation of Salmonella by the microbiological and PCR methods. The identification of colonies typical of Salmonella isolated by the microbiological method was carried out by mass spectroscopy. The conducted study demonstrated the indisputable advantages of the PCR method (after enriching air samples in BPW) over the classic microbiological method without enrichment for monitoring Salmonella in the air of poultry processing plants. The PCR method has a higher sensitivity and detection speed, allowing the pathogen to be detected even at low concentrations in a sample. This is especially important for monitoring areas with a potentially low microbial load, such as the final washing of broiler chicken carcasses. The microbiological method without the enrichment stage showed low detection of Salmonella in the study of 66.6 % of air samples (false negative results were obtained) of poultry processing plants and 80 % of air samples taken at the poultry farming enterprise. Increasing its sensitivity to a level comparable to the PCR method is possible only with the introduction of an additional enrichment step in a liquid non-selective nutrient medium, for example, in buffered peptone water. Thus, for prompt and reliable control of Salmonella contamination in the air, it is advisable to use the PCR method as the most rapid and sensitive tool, ensuring high reliability of results even with minimal bacterial contamination, and the microbiological method with sample enrichment as a relatively slow but reliable "golden" standard method.

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Introduction

At enterprises producing beef, pork and poultry meat, the air is acknowledged as a factor facilitating contamination of food products. In particular, slaughter shops are potentially critical as animals and poultry are the main source of microbial contamination of the air [1–3]. Analyses of the air at various sites of technological operations on slaughtering animals and poultry show similar trends in the microbial counts despite quantitative differences with lower levels before slaughter and higher values in the slaughter process both in "clean" (for example, at the site of accumulation of carcasses that are ready to chilling) and in "dirty" (bleeding) sites [4]. Processing of poultry meat includes the multistep sequence of operations, including slaughter, which consists in hanging, stunning, neck cutting and bleeding; dressing, which includes scalding, defeathering, head pulling, hock cutting, venting, evisceration, crop removal, neck cracking and cutting of neck flap; inside and outside washing of carcasses, decontamination and reprocessing; inspection of carcasses after slaughter, carcass chilling before cutting and packaging as described in [5] and GOST R 52469-2019[1].

As food safety has received high priority [6] and the industry has found itself under pressure to supply foods with minimal processing [7], fresher taste and appearance, with lower content of preservatives [8] and long shelf-life, it is necessary to apply an intervention strategy to control vectors of food contamination [9]. At food enterprises, bioaerosols can also be a potential factor influencing food safety and quality [10,11]. Practically all microorganisms in bioaerosols are easily transferred by air flows; however, their multiplication in the air is unusual due to the lack of moisture and nutrients. Despite sensitivity to the environmental conditions, foodborne pathogens can survive in the air combined, for example, with dust particles [12].

[1] GOST R 52469-2019 "Poultry processing industry. Poultry processing. Terms and definitions". Retrieved from https://docs.cntd.ru/document/1200167787. Accessed October 26, 2025

Due to the constant air movement in food enterprises, the control of the air environment is difficult, but the correct location of intake and exhaust air ducts, door openings and technological equipment optimizes the movement of air flows [13,14]. Periodical monitoring of the level of microorganisms in the air facilitates revealing potential sources of food contamination [15,16].

Air samples are analyzed using various methods: microscopic, microbiological, biochemical or molecular [17,18]. A choice of a method for air analysis for one or another indicator will depend on sensitivity and specificity of a method as well as on a method of air sampling [19,20]. It is necessary to choose a method for investigation before performing the procedure of air sampling.

Traditionally, culture-based methods are used in the food industry to quantify microorganisms in the air [21]. To reveal microbiota of the air environment, it is preferable to use different nutrient media to detect as many microbial species as possible [22,23]. A limitation of the culture-based microbiological method is the fact that with this approach only part of microbial population can be detected. For example, it is impossible to detect viable but non-culturable (VBNC) microorganisms using this method [24]. Despite this disadvantage, the microbiological method is a "golden" standard in the food microbiology.

Using phase contrast microscopy, it is possible to see both culturable and non-culturable forms of microorganisms in an air sample [25].

Molecular methods, such as 16S rDNA amplification by polymerase chain reaction (PCR) with the following sequencing and DNA-DNA hybridization, allow for increasing sensitivity and specificity with the simultaneous reduction of time necessary for analysis. For example, quantitative PCR (qPCR), which is successfully used in medical investigations for assessment of the total and species-specific airborne bacterial load, can also be used to control the air environment in food enterprises. Sensitivity of qPCR is higher by several orders of magnitude than that of the microbiological methods. Moreover, it is able to amplify DNA of VBNC cells [26]. At present, molecular methods are not used in the food industry as routine methods for air monitoring in facilities of food enterprises despite their advantages.

The aim of this research is to compare effectiveness of the microbiological and PCR methods for detection of *Salmonella* in the air at different technological sites of poultry processing plants.

Objects and methods

Objects of the research were air samples taken at different sites of the technological process in four poultry processing plants with complete or incomplete cycle of poultry slaughter and processing, as well as lairage zones.

— Enterprise No. 1: air samples were taken at the sites of raw material preparation ($n = 2$), species pre-packing, production of sausage products ($n = 2$), production of semi-finished products near the exit of products from a quick freezer, production of semi-finished products near the conveyer belt with nuggets after deep fat frying ($n = 2$), production of semi-finished products near the deep fat fryer, production of semi-finished products near the packaging machine ($n = 2$), packaging of finished products (frankfurt-type sausages), near the packaging machine, packaging of the finished products, near the heat shrink equipment, container washer, storage chamber for finished products;
— Enterprise No. 2: air samples were taken at the sites of evisceration, chilling, forming and packaging of semi-finished products, packaging of breaded culinary frozen products ($n = 2$), cutting of semi-finished products in pieces ($n = 2$), pre-packaging of dry auxiliary ingredients, container washer;
— Enterprise No. 3: air samples were taken at the sites of poultry hanging, bleeding, scalding and defeathering, evisceration and venting, spray cabinet, near the machine for leg processing, near the site of location of the machine for head pulling, air-water droplet spraying, Maestro, deboning of the thigh fillet, bath for by-products, container washing, machine deboning of breasts, accumulation of carcasses, final washing, carcass packaging;
— Enterprise No. 4: air samples were taken in the lairage zone, in the tunnel, in the gallery, location of the deboning cone, at the site of the line for further processing of thighs/breasts, on the line of modified atmosphere packaging (MAP), on the line of vacuum-packaging, in the buffer zone, on container washer, near the Morris water chiller, at the site of further processing of by-products. This is a poultry processing plant which carries out turkey slaughter and produces semi-finished products in pieces from turkey meat.

Air sampling was carried out during the working process using an air sampler Airwel (ALLIANCE BIO EXPERTISE, France) on the solid nutrient media: non-selective PCA (Plate Count Agar) (State Research Center for Applied Microbiology and Biotechnology (SRC AMB), Russia) and selective medium XLD (Xylose Lysine Deoxycholate) (SRC AMB, Russia). The volume of air samples was no less than 200 liters.

To detect *Salmonella*, two methods were used: microbiological and PCR.

Microbiological analysis of air samples collected on the non-selective PCA medium (SRC AMB) was carried out with enrichment in the buffered peptone medium (BPW) (SRC AMB, Russia) and of those collected on XLD agar without enrichment (SRC AMB, Russia). XLD agar was placed into an incubator (Binder BD240) (Binder, Germany) immediately after air sampling and incubated at a temperature of 37 °C for 24 hours. PCA agar with air samples was aseptically transferred from Petri dishes to homogenization bags, 100 cm^3 of BPW was added and homogenization was carried out. Then, incubation was carried out at a temperature of 37 °C for 24 hours.

After incubation, colonies characteristic of *Salmonella* were taken from XLD agar for the following identification.

In parallel, after incubation, 1 cm^3 of culture liquid was taken from BPW and transferred into liquid selective nutrient media: RV (Rappaport-Vassiliadis Broth) (SRC AMB, Russia) and MKTT (Müller-Kauffmann Tetrathionate Broth) (SRC AMB, Russia). Cultures were incubated at temperatures of 41.5 °C and 37 °C, respectively, for 24 hours. After that, cultures were transferred from each medium to the surface of XLD agar and incubation was carried out at a temperature of 37 °C for 24 hours. Colonies characteristic of *Salmonella* were transferred on TSA, incubated at a temperature of 37 °C for 24 hours and subjected to identification.

Identification was carried out by time-of-flight mass spectrometry (MALDI-TOF-MS) using a mass spectrometer Autof MS1000 (Autobio Diagnostics, China). To this end, bacterial mass of colonies was placed on a target plate and dried at room temperature. Then, 1.2 µl of formic acid was placed for 10 min. into each well with dried bacterial mass and dried, 1.2 µl of HCCA matrix (α-ciano-4- hydroxycinnamic acid, 99 %) was applied and dried again. The MALDI target was placed into the instrument and the equipment for microbial identification was started up using the software FlexControl (acquisition of spectra). Over several minutes, the software of the apparatus compared the obtained bacterial mass spectra of unknown microorganisms with spectra of identified microorganisms contained in the database of the instrument. During comparison based on the correlation of the obtained peaks and their intensity, matching scores were calculated: if the value was lower than 6.0, a result was considered unreliable and was not used in the subsequent work. A result was considered reliable and was taken into account at values of 6.0–9.0 at the genus level and 9.0–9.5 at the species level.

In parallel, bacteria of the genus *Salmonella* were detected by PCR from air samples after enrichment of PCA in BPW. DNA was isolated from 1 cm^3 of BPW using a set of reagents for the extraction of nucleic acids from animal biological samples (VECTOR BEST, Russia) with the Auto-Pure 4800 automatic nucleic acid extraction and purification instrument (Allsheng, China). PCR was performed on the instrument Fluorite (Xi'an TianLong Science and Technology Co., Ltd, China) using a kit for detection of *Salmonella* DNA "Reagent kit for detection of *Salmonella* spp. DNA by real-time PCR" (VECTOR BEST, Russia) according to manufacturer's instruction manual. Interpretation of the results was performed automatically by VECTOR BEST software (2025). All positive results were confirmed by the microbiological method[2].

[2] GOST 31659-2012 (ISO 6579:2002) "Food products. Method for detection of Salmonella bacteria". Retrieved from https://docs.cntd.ru/document/1200098239. Accessed October 26, 2025.

Results and discussion

Fifty two air samples were taken at the enterprises for slaughter and processing of broiler chickens ($n=3$) and turkey ($n=1$). The presence of *Salmonella* in them was determined using two methods described in the methods of research.

Salmonella were not detected by any of the used methods in the air samples taken at the following sites of enterprise No. 1: raw material preparation ($n=2$); species pre-packing; production of sausage products ($n=2$); production of semi-finished products, near the exit of products from a quick freezer; production of semi-finished products near the conveyer belt with nuggets after a deep fat fryer ($n=2$), near the deep fat fryer and packaging machine ($n=2$); packaging of finished products (frankfurt-type sausages), near the packaging machine, near heat shrink equipment; container washer; storage chamber for finished products.

Salmonella were absent in all air samples taken at different sites of enterprise No. 2: evisceration of carcasses; chilling of carcasses; cutting carcasses into semi-finished products ($n=2$); forming and packaging of semi-finished products; packaging of breaded culinary frozen products ($n=2$); prepackaging of dry auxiliary ingredients; container washer;

Also, *Salmonella* were absent in all air samples taken at enterprise No.4: in the lairage zone; in the tunnel; in the gallery; at the sites of location of the deboning cone and the line for further processing of thighs/breasts; on the line of modified atmosphere packaging (MAP); on the line of vacuum-packaging, in the buffer zone, on container washer, near the Morris water chiller, at the site of further processing of by-products.

In the air samples taken at enterprise No. 3, *Salmonella* were absent, but not at all sites. *Salmonella*-free sites included deboning of the thigh fillet and breasts, location of the bath for by-products; container washing; accumulator of carcasses and their package; the "dirty" zone, where machines for leg processing and head pulling from broiler chicken carcasses were located; application of air-water droplet spraying.

However *Salmonella* were detected in the air samples at several sites of enterprise No.3: carcass hanging, bleeding, scalding and defeathering of broiler chicken carcasses, evisceration and venting, at the site of location of Maestro/ pan conveyor and final washing of carcasses. Table 1. presents data obtained by different methods of analysis.

Table 1. Results of *Salmonella* detection in the air samples taken at poultry processing enterprise No.3 and analyzed by different methods

Sites at enterprise No.3	Presence of *Salmonella* spp.		
	Microbiological method		PCR method
	after enrichment	without enrichment	
Hanging	detected	detected	detected
Bleeding	detected	not detected	detected
Scalding / defeathering	detected	detected	detected
Evisceration / venting	detected	not detected	detected
Maestro/ pan conveyor	detected	not detected	detected
Final washing	detected	not detected	detected

Salmonella were detected in all six samples (100 %) by the PCR method and microbiological method after enrichment of the air samples. Without enrichment, *Salmonella* were detected only in two samples (at the sites of hanging and scalding / defeathering) out of six.

Following the aim of the research and based on the results obtained, including those presented in Table 1, we can conclude that PCR and microbiological methods used with the pre-enrichment of the air samples in BPW are significantly superior. With such an approach *Salmonella* were detected in 100 % of analyzed samples. Comparative analysis of two approaches of the microbiological method revealed an importance of the enrichment stage when detecting *Salmonella* in the air. Presumably, the microbiological method without enrichment of the air samples is not effective due to the low level of air contamination with *Salmonella*, which can vary depending on a processing object (live poultry, carcasses, meat).

It is possible that the reason for not detecting *Salmonella* in the air samples at enterprise No.1 even with the use of the sensitive PCR method is the fact that this enterprise does not perform poultry slaughter, which is a process with a high risk of contamination of the air environment. Processing of chilled or frozen raw materials (dressing, forming, frying and packaging), which is carried out at this enterprise carry insignificant risk of active entry of *Salmonella* into the air. In case of their transfer to the air, they can be undetected depending on the sensitivity of the method.

At enterprise No. 2, air samples were taken at the sites with a high risk of contamination, namely evisceration and chilling of carcasses, as well as at the sites with medium and low risk of contamination — production and packaging of semi-finished products, respectively. The targeted sampling at the first two sites was determined by the fact that poultry intestine is the main reservoir of *Salmonella* [27], which creates an increased probability of their entry into the air during technological operations. Air samples at the sites of production and packaging of semi-finished products were used to assess the sensitivity of the methods. Comparative analysis of air samples taken at these critically important points allows for more objective assessment of the effectiveness of PCR and microbiological methods in the conditions of the real contamination load. However, *Salmonella* were absent in the air samples taken at all sites, including at the site of evisceration. This is possible when the integrity of the gastro-intestinal tract is not violated and, as a consequence, there is a low level of air contamination, which is lower than sensitivity even of the PCR method.

For valid comparative assessment of the methods for *Salmonella* detection, it was necessary to ensure conditions at which the concentration of targeted microorganisms in samples would reliably exceed the threshold of the sensitivity of methods. This is what determined the extended sampling at enterprise No. 3, which included all sites of the cycle of poultry slaughter and primary processing (poultry hanging, poultry bleeding, carcass scalding, carcass defeathering) contrary to enterprises No.1 and No. 2. The choice of these zones was not accidental, as they are characterized by the maximum microbial load, including *Salmonella*, which is confirmed by both the data of monitoring of the objects of the production environment (not presented in the paper) and the results of the analysis of the air by foreign colleagues [28]. Therefore, sampling at zones with undoubtedly high contamination enabled creating a representative model for the comparative analysis of effectiveness of the PCR and microbiological methods. *Salmonella* were detected in the air samples taken in these zones as well as in the zones of carcass evisceration, pan conveyor, application of air-water droplet spraying and final washing. In [29], an increase in the percent of broiler carcass contamination (from 10 to 40 %) was observed during evisceration and spray washing after evisceration; with that, *Salmonella* counts increased from 3.9 to 5.1 log CFU/carcass. Apparently, detection of *Salmonella* on broiler chicken carcasses after washing can also be explained by the presence of flagella in *Salmonella*– a key factor of adhesion to the skin of broilers [30].

Comparative analysis revealed key technological operations that present a risk of air contamination. This is indicated by the detection of *Salmonella* in the air at the evisceration site and, what is especially indicative, after final washing of carcasses, which was confirmed by the used methods. At the same time, at the sites of leg processing, head pulling and production of semi-finished products (enterprise No. 3), neither PCR nor the microbiological method revealed *Salmonella* in the air. The most significant for comparison of the methods for *Salmonella* detection is divergence of the results at the identical sites (evisceration and chilling) of enterprises No. 2 and No. 3. At enterprise No. 3, *Salmonella* in the air of these zones were detected, while at enterprise No. 2 they were not. This contradiction revealed by both methods indicates that a level of air contamination depends not only on the type of operations but also on other factors. For example, Ferguson et al. [31] stated that effectiveness of sanitary measures and initial contamination of raw materials can be such factors. The air represents the main hazard, being the main route of transfer of contaminants [32] and acting as a key vector for bioaerosols — suspended particles containing bacteria, mold spores and yeasts. Settling on products and equipment surfaces, bioaerosols create a direct risk of microbiological contamination [31]. Contamination at the stage of poultry processing can occur from multiple sources: production environment of a shop, poultry itself, equipment and personnel. These contaminants can have physical, chemical or biological nature [33].

At enterprise No. 4, which like enterprise No. 3 is a poultry processing plant of the whole cycle of slaughter and processing, but only of turkeys, *Salmonella* were not detected in any air samples by either the microbiological method (with and without enrichment) or by PCR. Consistent negative results obtained by both methods prompt

to propose several assumptions about reasons for *Salmonella* absence in the air of this enterprise that were corroborated upon the detailed analysis of the enterprise. The absence of *Salmonella* in the air of this enterprise was a consequence of the complex of measures, such as prevention at a level of raw materials, when the strict veterinary control is carried out, and work is performed with certified enterprises supplying *Salmonella*-free poultry; technological solutions that consist in automation of slaughter and evisceration processes minimizing formation of aerosols, as well as the use of effective ventilation system supplied with bactericidal lamps; organizational and hygienic measures in a form of the verified procedure of sanitary treatment of equipment, clear zoning and logistics of flows of raw materials, finished products and personnel. Therefore, the complex approach at enterprise No. 4 allowed for creating conditions under which a level of air contamination with *Salmonella* was lower that the limit of detection even for a highly sensitive method such as PCR, which is corroborated by consistent results of both methods and emphasizes an importance of preventive measures.

In addition, analysis was carried out for air samples ($n = 22$) taken randomly in one of the facilities of the poultry feeding station where pre-slaughter holding of broiler chickens is performed. From this enterprise, broiler chickens are sent to poultry processing enterprise No.3, where *Salmonella* were detected in the air samples. The results are presented in Table 2.

Table 2. Results of *Salmonella* detection in the air samples of the facility of the poultry feeding station, where pre-slaughter holding of broiler carcasses is performed

Air samples	Presence of Salmonella spp.		
	Microbiological method		PCR after enrichment
	after enrichment	without enrichment	
1.	not detected	not detected	detected
2.	not detected	not detected	not detected
3.	not detected	not detected	not detected
4.	not detected	not detected	not detected
5.	not detected	not detected	not detected
6.	detected	not detected	detected
7.	detected	not detected	detected
8.	detected	not detected	detected
9.	not detected	not detected	not detected
10.	not detected	not detected	not detected
11.	detected	detected	detected

Data on air contamination with *Salmonella* were obtained for the facility where poultry were kept before slaughter. Similar to the previous investigation, the results obtained by the microbiological and PCR method after sample enrichment had the 100 % agreement of the results. With that, for one sample, the 100 % agreement was obtained for all three approaches. These approaches detected *Salmonella* in 45.5 % of the analyzed air samples. However, detection rate of *Salmonella* in the air samples by the microbiological method without enrichment was no more than 9.1 %.

As a result of comparative analysis of the methods (PCR and microbiological method) and approaches (with and without enrichment) for *Salmonella* detection in the air of poultry processing plants and one poultry farming enterprise, key differences in their effectiveness were established. The PCR method demonstrated 100 % detection rate of *Salmonella* after enrichment of the air samples, which is in agreement with other studies indicating that enrichment of samples in BPW for 18–24 hours with the following detection of *Salmonella* by the PCR method can improve their detection [34,35]. This method enables detecting both viable and injured microbial cells. Contrary to this method, the microbiological method can detect only viable and culturable *Salmonella* cells both without enrichment, for example in areas with a high level of contamination, and with enrichment even in areas with a low level of contamination. The study confirmed that the main risks of air contamination occur at the stages of slaughter and primary processing at the hanging sites where due to stress poultry make intense movements and feathers, down hair and dust from their surfaces contaminated with *Salmonella* enter the air; at the sites of scalding and defeathering due to formation of bioarosols; at the sites of evisceration due to the rupture of the intestine and at the sites of final washing due to cross-contamination via air flows. From poultry farming enterprises, however, birds come for slaughter being already contaminated with *Salmonella*.

Thus, the differences in the results obtained on the contamination of the air between enterprises were determined not by the errors of these methods but by the different levels of biosecurity at these enterprises. Therefore, the differentiated approach can be recommended to effectively monitor the air in production facilities of poultry processing plants: the PCR method with the pre-enrichment of air samples for the operative control of the air at all sites especially after sanitary treatment and the microbiological method with enrichment within the framework of the production control as well as for confirmation of positive results obtained by the PCR method and acquisition of the native culture for serotyping of *Salmonella* circulating in an enterprise. According to an opinion of several authors, traditional culture-based methods have limitations compared, for example, with the method of high-precision sequencing CRISPR-SeroSeq as they are based on isolation of several colonies and consequently underestimate the diversity of serovars. Combination of selective pre-enrichment with the molecular method in the environmental samples demonstrated comparable isolation of serovars in comparison with the traditional enrichment reducing at the same time the isolation process by 24 hours [36]. However, when monitoring pathogens in the air, it is necessary to assess viability of microorganisms to reveal whether they present a threat to human or poultry health. It is recommended to combine methods, both culture-dependent and culture-independent (for example, PCR), to prevent false negative results of detection of pathogens [37]. Control of the air environment, especially

at poultry farming enterprises, is of utmost importance. Contrary to common beliefs about oral transmission of the microorganism, transfer of *Salmonella*, in particular *S.* Enteritidis, can occur by the airborne route, and an impetus of this event can be induced molting of poultry [38]. Molecular methods detect microorganisms irrespective of their viability, which leads to overestimating their concentration in the air [39]. When using the microbiological method, it is recommended to combine several selective nutrient media, for example, Brilliant Green agar, Modified Lysine Iron agar and XLT4. With that, it is important to choose an effective method for air sampling, which can ensure a sensitive alternative to the traditional method for detection of this pathogen in the poultry environment [40].

Conclusion

The performed research demonstrated undeniable advantages of the PCR method (samples after enrichment) compared to the classic microbiological method without enrichment for *Salmonella* monitoring in the air environment of poultry processing plants. The PCR method has higher sensitivity and speed of detection, making it possible to detect the pathogen even at a low concentration in a sample. This is especially important for the control at the stages of potentially low microbial load, such as the site of final washing of broiler chicken carcasses. The microbiological method without the enrichment stage showed low detection rate. False negative results were obtained in 66.6 % of air samples when analyzing the air in the poultry processing plants and 80 % of air samples taken in the poultry farming enterprise. Increasing its sensitivity up to a level comparable with the PCR method is possible only upon introduction of the additional stage of enrichment in the liquid non-selective nutrient medium. Therefore, for rapid and reliable control of contamination of the air environment with *Salmonella*, it is advisable to use the PCR method as the most rapid and sensitive tool that ensures high reliability of results even at minimal bacterial contamination and the microbiological method with enrichment of samples as a relatively slow but reliable "golden" standard method.

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AUTHOR INFORMATION

Dagmara S. Bataeva, Candidate of Technical Sciences, Leading Research Fellow, Department of Hygiene of Production and Microbiology, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhin str., 109316, Moscow, Russia.
E-mail: d.bataeva@fncps.ru
ORCID: https://orcid.org/0000-0002-1374-2746
* corresponding author

Yulia K. Yushina, Doctor of Technical Sciences, Head of Department of Hygiene of Production and Microbiology, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhin str., 109316, Moscow, Russia.
E-mail: yu.yushina@fncps.ru
ORCID: https://orcid.org/0000-0001-9265-5511

Anastasia A. Semenova, Doctor of Technical Sciences, Professor, Deputy Director, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhin str., 109316, Moscow, Russia. E-mail: a.semenova@fncps.ru
ORCID: https://orcid.org/0000-0002-4372-6448

Maria A. Grudistova, Candidate of Technical Sciences, Researcher, Department of Hygiene of Production and Microbiology, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhin str., 109316, Moscow, Russia.
E-mail: m.grudistova@fncps.ru
ORCID: https://orcid.org/0000-0002-8581-2379

Elena V. Zaiko, Candidate of Technical Sciences, Department of Hygiene of Production and Microbiology, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhin str., 109316, Moscow, Russia. E-mail: e.zaiko@fncps.ru.ru
ORCID: https://orcid.org/0000-0002-5048-9321

Anzhelica A. Makhova, Researcher, Department of Hygiene of Production and Microbiology, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhina str., 109316, Moscow, Russia. E-mail: a.mahova@fncps.ru
ORCID: https://orcid.org/0000-0002-2508-2888

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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