UDC 619:579:614.48:631.223.6:636.4.084.7(477) DOI 10.36016/JVMBBS-2024-10-3-6

MICROBIAL LOAD OF FACILITIES FOR KEEPING PIGS OF DIFFERENT PRODUCTION GROUPS

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Summary. The study analyzed the microbial load of objects in the facilities where pigs of different production groups were kept at the final stage of production cycles, immediately before disinfection measures. The study found that the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in the swabs from the surfaces of the studied objects varied from 5.00 to 6.88 log CFU/cm³. The lowest quantity of bacteria was found on drinkers and feeders, while the highest quantity was on the facilities' floor. The average level of microbial load in the facilities for keeping sows, farrowing, and growing piglets ranged from 5.91 to 6.07 log CFU/cm³. The highest values were observed for the study of swabs taken in the piglet-rearing facility. The proportion of field isolates of the rod, cocci, and spiral shapes of microorganisms in the rearing facility was 62.1%, 28.8%, and 9.1%, respectively, in the farrowing facility — 63.9%, 29.2%, and 6.9%, and in the sow housing facility — 66.2%, 26%, and 7.8%. *Escherichia coli* was dominant in the rearing facility — 13.9% of isolates, *Proteus mirabilis*, *Bacillus subtilis*, and *Campylobacter jejuni* — 9.7% each, and *Citrobacter freundii*, *Enterococcus faecalis*, and *Enterococcus faecium* — 8.3% each. In farrowing facilities, the proportion of *E. coli* isolates was 16.6%. 7.5% fewer isolates belonged to *B. subtilis*, *Streptococcus salivarius*, and *C. jejuni*, and 9% fewer isolates belonged to *Klebsiella pneumoniae*, *P. mirabilis*, *E. faecalis*, and *E. faecium*. In the sow housing facility, the proportion of *E. coli* isolates was 12.9%, the number of *P. mirabilis* isolates was 1.2% less, and *C. freundii* was 3.8% less

Keywords: MAFAnM, contamination, disinfection

Introduction. Pig farming is one of the most important livestock industries and plays an important role in meat production. For the efficient production of pork, it is important to establish and maintain proper sanitary and hygienic conditions in pig housing facilities. Among these conditions, the microbial load of livestock facilities deserves special attention as it has a major impact on animal health, productivity, and product quality (Haidukevych and Semenova, 2023; Kot et al., 2019; Myronchuk and Peleno, 2023).

It is known that the microbiocenosis of surfaces with which animals come into contact influences the development of infectious diseases, the state of the animals' immune system, and their general physiological condition (Rudenko et al., 2021).

Exceeding permissible standards for the number of microorganisms in the air is often an etiological factor in developing respiratory diseases, which are one of the most common problems in pig farms (Bolibrukh and Rublenko, 2023; Luyckx et al., 2016).

Changes in the microbial load of facilities can affect the metabolism of pigs and the occurrence of infectious diseases, resulting in decreased weight gain, increased production costs, and deterioration of meat quality (Trinh et al., 2018).

According to Wen et al. (2021), the species and quantitative composition of the indoor microflora can vary significantly at different stages of the production cycle. The factors that cause these changes can be the number of animals kept in a given facility, their density, temperature, humidity, disinfection quality, ventilation, etc. (Buoio et al., 2023; Wen et al., 2021).

In modern pig production, it is important to identify and eliminate potential risks and implement effective measures to minimize the microbial load of the facilities. These tasks are usually accomplished through regular monitoring of the microbial load of the air in the facilities where the animals are kept and of the surfaces with which they come into contact, as well as through high-quality disinfection, the introduction of modern ventilation systems on the farms, temperature, and humidity control, etc. (Luiken et al., 2020).

Since the creation of optimal conditions for the keeping of pigs, taking into account the microbial load of the facilities for their keeping reduces the risks of occurrence and development of diseases, improves the general physiological condition of animals, increases their productivity and economic performance of enterprises, the planned research is relevant.

The study aimed to investigate the total microbial load and the species composition of the microflora of farrowing, piglets rearing, and sow housing facilities at the end of each production cycle.

Materials and methods. The experiments were conducted at the LLC 'Eco Meat', which was established in October 2013 with the support of Polish partners. It is located in the village of Batiatychi, Lviv District, Lviv Region. The farm has a total capacity of 3,200 sows. The animals are kept in two farrowing facilities, six piglet-rearing facilities, two rooms for growing animals, and

two rooms for keeping single and farrowing sows. The piglets are kept in the farrowing room until they are 28 days old, and in the rearing room from 28 to 63 days old, after which they are sold to other farms. At the end of each stage, the farm is disinfected by spraying with 'Vulkan Max' (Huvepharma, France).

The material for the study were swabs taken at the end of the production cycle, immediately before disinfection, from the floor, feeders, drinkers, walls, and cage partitions in the piglet rearing, sow housing, and farrowing rooms, five samples from each facility. Sampling to determine the type and total contamination of livestock facilities with mesophilic aerobic and optionally anaerobic microorganisms (MAFAnM) was performed according to the 'Recommendations for the Sanitary and Microbiological Examination of Swabs from the Surfaces of Test Objects and Objects of Veterinary Surveillance and Control' (Yakubchak et al., 2005). The total contamination was determined by the amount of MAFAnM in the swabs and expressed as log CFU/cm^3.

Special and selective media were used to cultivate field isolates. Microorganisms of the Enterobacteriaceae family were cultured on Endo agar (HiMedia, Germany), *Pseudomonas aeruginosa* on Cetrimide Agar (Merck, Germany). For *Staphylococcus aureus*, salt agar for the isolation of staphylococci (Farmaktiv, Ukraine), *Streptococcus salivarius* — blood agar (Merck, Germany), and for *Enterococcus faecalis* and *Enterococcus faecium* — Enterococcus agar (Farmaktiv, Ukraine) were used. Campylobacter selective agar (HiMedia, Germany) was used for *Campylobacter jejuni*. The spore-forming microorganisms *Bacillus subtilis* and *Bacillus megaterium* were cultured on nutrient agar with subsequent identification by the ability to hydrolyze pectin. *Clostridium perfringens* was cultured under anaerobic conditions using Kitt-Tarozzi medium (Conda, Spain) (Scully and Orlygsson, 2023).

Field isolates were identified based on the study results of morphological, tinctorial, cultural, and biochemical properties under the following regulatory documents: ISO 10272-1:2017 Microbiology of the Food Chain — Horizontal Method for Detection and Enumeration of *Campylobacter* spp. — Part 1: Detection Method (ISO, 2017a), ISO 21528-1:2017 Microbiology of the Food Chain — Horizontal Method for the Detection and Enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae (ISO, 2017b), ISO 15213-2:2023 Microbiology of the Food Chain — Horizontal Method for the Detection and Enumeration of *Clostridium* spp. — Part 2: Enumeration of *Clostridium perfringens* by Colony-count Technique (ISO, 2023), ISO 7932:2004 Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Presumptive *Bacillus cereus* — Colony-count Technique at 30 Degrees C (ISO, 2004), ISO 13720:2010 Meat and Meat Products — Enumeration of Presumptive *Pseudomonas* spp. (ISO, 2010), ISO 16266:2006 Water Quality — Detection and Enumeration of *Pseudomonas aeruginosa* — Method by Membrane Filtration (ISO, 2006), and Bergey's Manual of Systematic Bacteriology (Garrity et al., 2005a, 2005b).

The obtained numerical values were statistically processed using the program Statistica ver. 10.0 (StatSoft, USA) with the determination of the arithmetic mean (M) and its error (m). The reliability of the results was assessed by the Student's test.

Results and discussion. Determination of the microbial load of objects before disinfection allows us to estimate the level of contamination and to choose the most effective disinfection measures. From the results presented in Fig. 1, it can be seen that after the technological process was completed, the amount of MAFAnM in the farrowing, piglet rearing, and sows' housing facilities ranged from 5.00 to 6.88 log CFU/cm^3 of the swab.

The lowest number of bacteria was on the surface of drinkers and feeders and ranged from 5.00 to 5.20 and 5.28 to 5.65 log CFU/cm^3 of the swab, respectively. On the walls of the facilities, the number of mesophilic aerobic and facultative anaerobic microorganisms ranged from 6.10 to 6.24 log CFU/cm^3 of swab, and on plastic partitions — from 6.23 to 6.41 log CFU/cm^3 of swab. The highest level of bacteria was recorded in the swabs taken from the floor — from 6.78 to 6.88 log CFU/cm^3 of the swab.

Comparing the microbial contamination of the studied objects, it was found that drinking bowls and feeders were the least loaded with microorganisms in the facility intended for farrowing sows, and the most — for rearing piglets. The number of MAFAnMs on their surfaces was 5.00 and 5.16 and 5.20 and 5.65 log CFU/cm^3, respectively. On the walls and plastic intercellular partitions, the lowest number of bacteria, 6.10 and 6.23 log CFU/cm^3 of the swab was in the sow housing facility, and the highest number, 6.24 and 6.41 log CFU/cm^3 of the swab was in the piglet rearing facility. The floor, drinking bowls, and feeders were the least contaminated with microflora in the piglet rearing facility, and the most — in the sow housing facility, and the number of MAFAnMs was 6.78 and 6.88 log CFU/cm^3 of the swab, respectively.

Compared to the least microbially loaded objects, which in all studied facilities were drinkers, on the surface of feeders, walls plastic partitions, and floors, the number of MAFAnMs in the farrowing facility was 5.6%, 23.4%, 26.4%, and 35.6% higher, respectively, in the piglet rearing facility by 8.6%, 20.1%, 23.3%, and 31.3%, and in the sow housing facility by 3.3%, 18.2%, 20.7%, and 33.3%.

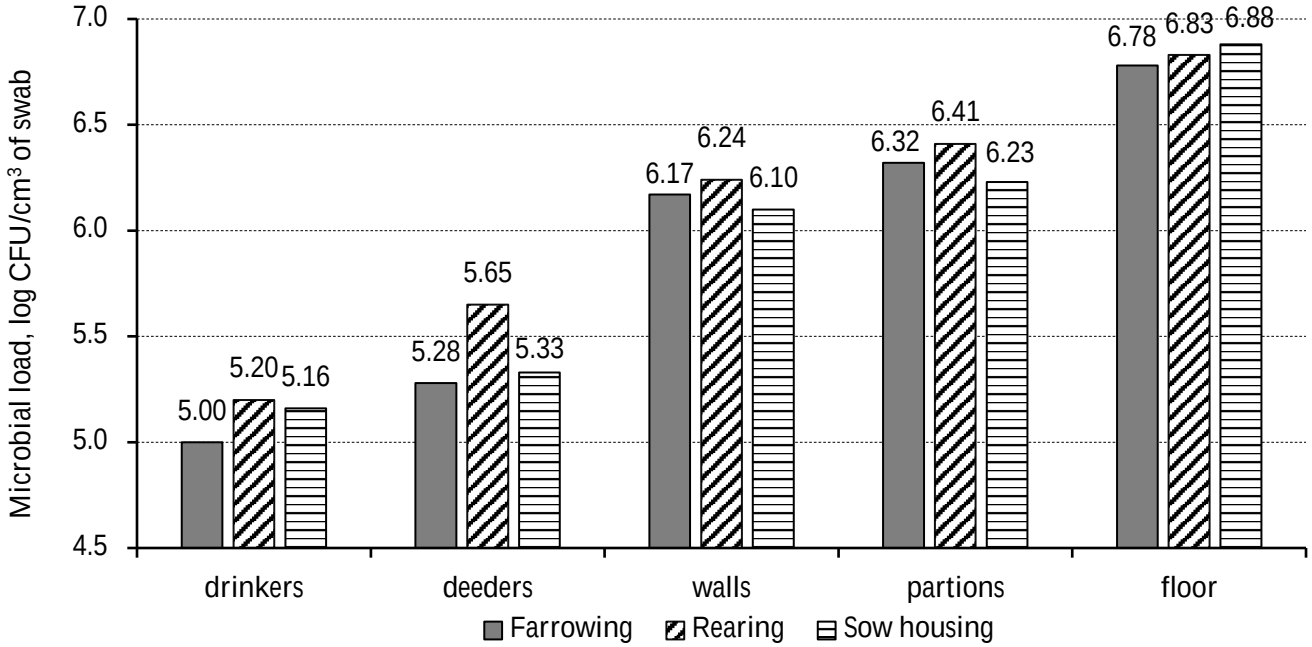

Figure 1. Microbial load of farrowing, piglet rearing, and sow housing facilities after the end of the production cycle.

From the data shown in Fig. 2, it can be seen that the lowest average level of microbial load was observed in the farrowing facility and amounted to 5.91 log CFU/cm^3 of the swab, slightly higher in the sow housing facility (5.94 log CFU/cm^3 of the swab), and the highest in the growing facility (6.07 log CFU/cm^3). The data obtained are consistent with the results obtained by other researchers (Shkromada, 2014).

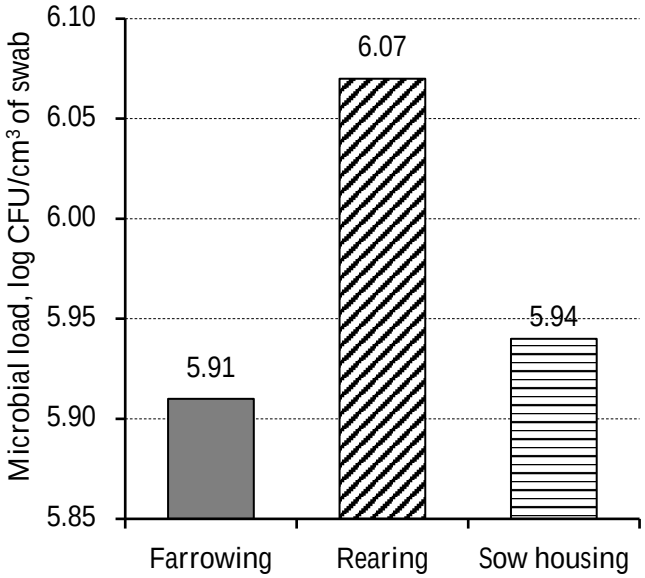

Figure 2. Average level of microbial load in farrowing, rearing, and sow housing facilities.

Analyzing the species composition of microorganisms isolated from the swabs taken from the facilities for keeping and farrowing sows and rearing piglets (Table 1), it was found that the microbiocenosis was formed by field isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, *Bacillus subtilis*, *Bacillus megaterium*, which have a rod-shaped form, *Staphylococcus aureus*, *Streptococcus salivarius*, *Enterococcus faecalis*, *Enterococcus faecium*, which belong to cocci, and *Campylobacter jejuni*, which belong to the spiral shape.

Table 1 — Characteristics of the species composition of microflora of facilities for keeping pigs of different production groups (n = 215)

Species of micro-organisms	Number of isolates					
	rearing		farrowing		sow housing	
	Abs.	%	Abs.	%	Abs.	%
E. coli	10	13.9	11	16.6	10	12.9
K. pneumoniae	5	6.9	5	7.6	4	5.2
C. freundii	6	8.3	4	6.1	7	9.1
P. mirabilis	7	9.7	5	7.6	9	11.7
P. aeruginosa	4	5.7	3	4.5	5	6.5
C. perfringens	5	6.9	3	4.5	5	6.5
B. subtilis	7	9.7	6	9.1	6	7.8
B. megaterium	2	2.8	4	6.1	5	6.5
S. aureus	5	6.9	3	4.5	6	7.8
S. salivarius	4	5.7	6	9.1	3	3.9
E. faecalis	6	8.3	5	7.6	6	7.8
E. faecium	6	8.3	5	7.6	5	6.5
C. jejuni	5	6.9	6	9.1	6	7.8

The lowest number of microbial isolates was identified in the farrowing facility. Of the 66 field isolates, 41 (or 62.1%) were rod-shaped, 19 (or 28.8%) were spherical, and 6 (or 9.1%) were spiral. A total of 72 field isolates were isolated in the piglet-rearing facility. Of these, 46 (63.9%) were rod-shaped (*E. coli*, *K. pneumoniae*, *C. freundii*, *P. mirabilis*, *P. aeruginosa*, *C. perfringens*, *B. subtilis*, *B. megaterium*), 21 (29.2%) were spherical (*S. aureus*, *S. salivarius*, *E. faecalis*, *E. faecium*), and 5 (6.9%) were spiral (*C. jejuni*).

The largest number of microorganisms (77) was isolated from the swabs taken from the sow housing facility. This number was 6.9% and 11.7% higher than the number of isolates from the piglet-rearing and farrowing facilities. At the same time, rod-shaped microorganisms (*E. coli*, *K. pneumoniae*, *C. freundii*, *P. mirabilis*, *P. aeruginosa*, *C. perfringens*, *B. subtilis*, *B. megaterium*) were represented by 51 field isolates, which amounted to 66.2%, spherical (*S. aureus*, *S. salivarius*, *E. faecalis*, *E. faecium*) — 20 field isolates (26.0%), and spiral (*C. jejuni*) — 6 field isolates (7.8%).

In all the studied facilities, the dominant number of field isolates was identified as *E. coli*. Their number in the farrowing facility was 16.6%, in the piglet rearing facility — 16.9% and in the sow housing facility — 12.9%. In the piglet rearing facility, the number of field isolates of *P. mirabilis*, *B. subtilis*, and *C. jejuni* was 4.2% less than *E. coli; C. freundii*, *E. faecalis*, and *E. faecium* — 5.6 %*; K. pneumoniae*, *C. perfringens*, and *S. aureus* — 7.0%*; P. aeruginosa* and *S. salivarius* — 8.2%*;* and *B. megaterium* — 11.1%. In the farrowing facility 9.1% of field isolates belonged to each of *B. subtilis*, *S. salivarius*, and *C. jejuni*; 7.6% to each of *K. pneumoniae*, *P. mirabilis*, *E. faecalis*, and *E. faecium;* 6.1% to each of *C. freundii* and *B. megaterium;* 4.5% to each of *P. aeruginosa*, *C. perfringens*, and *S. aureus.*

The difference compared to *E. coli* was 7.5%, 9.0%, 10.5%, and 12.1%, respectively. In the sow housing facility, as well as in the piglet-rearing facility, the largest number of field isolates, after *E. coli*, belonged to *P. mirabilis*, and the difference was only 1.2%. The third, by the number of field isolates, was *C. freundii*, the fourth — *B. subtilis*, *S. aureus*, *E. faecalis*, and *C. jejuni*, the fifth — *P. aeruginosa*, *C. perfringens*, *B. megaterium*, and *E. faecium*, the sixth — *K. pneumoniae* and the seventh — *S. salivarius*, which accounted for 9.1%, 7.8%, 6.5%, 5.2%, and 3.9% of field isolates, respectively, and the difference compared to *E. coli* was 3.8%, 5.1%, 6.4%, 7.7%, and 9.0%.

Thus, the study of the microbial load of objects in the facilities for keeping pigs of different production groups showed that the number of MAFAnM on the floor, partitions, walls, feeders, and drinkers ranged from 5.00 to 6.88 log CFU/cm^3 of the swab. The highest microbial load was in the piglet rearing room and amounted to 6.07 log CFU/cm^3 of the swab. Similar results were obtained by Scicchitano et al. (2024), who studied the spread of antimicrobial-resistant bacteria on pig farms and in the environment, and Luyckx et al. (2016) when studying the bacterial load in pig nurseries where the objects for research were synthetic mesh, concrete wall, synthetic wall, drinkers, and feeders.

The established values of the microbial load meet the sanitary and hygienic requirements for livestock premises (Nebylytsia et al., 2023), and the microbial load from 6.78 to 6.88 log CFU/cm^3 of the swab on the floor of the studied facilities indicates the need for increased attention to the sanitation of floors and surfaces in such facilities (MHU, 2023).

As a result of identification of 215 field isolates by morphological, tinctorial, cultural, and biochemical properties, 138 of them were rod-shaped (*E. coli*, *K. pneumoniae*, *C. freundii*, *P. mirabilis*, *P. aeruginosa*, *C. perfringens*, *B. subtilis*, *B. megaterium*), 60 spherical (*S. aureus*, *S. salivarius*, *E. faecalis*, *E. faecium*), and 17 spiral (*C. jejuni*). Similar microorganisms have been isolated from the pig pen premises (Shkromada, 2014; Shkromada and Hrek, 2022). These data emphasize the importance of regularly monitoring microbial forms for rapid response in case of detection of pathogenic microorganisms.

The division of field isolates into Gram-positive and Gram-negative microorganisms is justified because gram-positive microorganisms have a thick layer of peptidoglycan in the peptide wall, which provides them with additional protection against physical and chemical factors and, in addition, they can produce special cryptoproteins that help to withstand environmental stresses (Xue, 2020).

The microorganisms we isolated belonged to aerobes (86.05%), anaerobes (6.05%), and 7.90% to microaerophiles, which were represented by 17 field isolates of *C. jejuni*, which is consistent with the studies of Zhu et al. (2019).

Other researchers (Ferone et al., 2020; Fischer et al., 2016) have isolated microorganisms similar to those we identified in terms of cultural and biochemical properties in swabs taken from the facilities of a pig farm.

Thus, before the sanitation measures, the pig housing facilities were contaminated with *E. coli*, *K. pneumoniae*, *C. freundii*, *P. mirabilis*, *P. aeruginosa*, *C. perfringens*, *B. subtilis*, *B. megaterium*, *S. aureus*, *S. salivarius*, *E. faecalis*, *E. faecium*, *C. jejuni*, which poses a risk to animal health and economic efficiency of production, requiring regular microbial monitoring and controlled disinfection.

Conclusions. 1. After completion of the technological process, the number of MAFAnM on the floor, partitions, walls, feeders, and drinkers in the farrowing facility was in the range of 5.00 to 6.78 log CFU/cm^3 of the swab, in the piglet rearing facility — from 5.20 to 6.83 log CFU/cm^3

of the swab and in the sow housing facility — from 5.16 to 6.88 log CFU/cm^3 of the swab.

2. The highest average microbial load was found in the piglet rearing facility (6.07 log CFU/cm^3 of the swab), while it was 2.14% and 2.64% lower in the sow housing and farrowing facilities, respectively.

3. The lowest number of bacteria was on the surface of drinkers and feeders (5.00–5.65 log CFU/cm^3 of the swab), the average number was on the walls of the facilities and plastic partitions (6.10–6.41 log CFU/cm^3 of the swab), and the highest number was in the swabs taken from the floor (6.78–6.88 log CFU/cm^3 of the swab).

4. The microbiocenosis of the studied facilities was formed by rod-shaped forms (*E. coli*, *K. pneumoniae*, *C. freundii*, *P. mirabilis*, *P. aeruginosa*, *C. perfringens*, *B. subtilis*, *B. megaterium*), spherical forms (*S. aureus*, *S. salivarius*, *E. faecalis*, *E. faecium*) and spiral forms (*C. jejuni*), the proportion of which in the facility for rearing young animals was 62.1%, 28.8%, and 9.1%, in the farrowing facility — 63.9%, 29.2%, and 6.9%, and in the sow housing facility — 66.2%, 26.0%, and 7.8%, respectively. The dominant species in the piglet rearing facility were *E. coli* — 13.9% of field isolates, *P. mirabilis*, *B. subtilis* and *C. jejuni* — 9.7%, and *C. freundii*, *E. faecalis*, and *E. faecium* — 8.3%. In farrowing facilities, the number of isolated *E. coli* was 16.6%, which is 7.5% fewer than the number of isolates belonging to *B. subtilis*, *S. salivarius*, and *C. jejuni*, and 9.0% fewer than *K. pneumoniae*, *P. mirabilis*, *E. faecalis* and *E. faecium*. In the sow housing facility, the number of *E. coli* isolates was 12.9%, the number of *P. mirabilis* isolates was 1.2% lower, and the number of *C. freundii* isolates was 3.8% lower.

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