Article
https://doi.org/10.61900/SPJVS.2023.01.06

ASSESSMENT OF THE SPOILAGE MICROFLORA IN SWINE AND BROILER CARCASSES

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Abstract

The microbial load is of major importance in terms of the quality, sanity, and freshness of the meat. The aim of our study was to perform a microbial risk assessment at warm and chilled swine and broilers carcasses represented by the psychrotrophic bacteria. The research material was represented by swine and broiler carcasses collected in past years. The results showed a variation in swine and broilers microbiological carcasses. The point of interest was based on the microorganisms presented in both species. Microbial load from the surface of carcasses is significantly influenced by the temperature in the chilling room of the slaughterhouse, if the temperature is inadequate, the microbial load is significantly higher.

Key words: Microbial residues, consumer safety, sustainable environment

Microorganisms, by their characteristics, can reduce the quality of food, or even make it inedible, either through their pathogenic action, or through degradation and the production of toxic metabolite.

Psychrotrophic bacteria produce different types of spoilage depending on the conditions of keeping the meat and its age. The spoilage microflora, in general, multiplies faster at low temperatures than the pathogenic one, with which it is otherwise in competition. That is why in most cases, at the level of meat kept at refrigeration temperatures, signs of spoilage appear before the number of pathogenic germs is harmful to the consumer, but this is not true in all cases, which is why the lack of organoleptic changes, not it is synonymous with the lack of harmfulness (Belous, 2023)

The microbial population at the surface of swine carcasses before chilling was represented by the following genera: *Staphylococcus, Micrococcus, Lactobacillus, Neisseria, Aeromonas, Acinetobacter, Moraxella, Pseudomonas, Yersinia, Serratia, Hafnia, Proteus* and *Escherichia*.

The load and the initial configuration of the microflora at the broiler carcass level is influenced by the animal's health condition before slaughter, the duration, and conditions during transport, as well as by the way the carcasses are processed in the slaughterhouse. Therefore, the results obtained regarding the load and the microbial configuration on the surface of the carcasses are largely dependent on the strict observance of hygiene rules along the entire technological flow of carcass processing. Most important microbial population detected at surface of the broiler carcasses before chilling was represented by following genera: *Pseudomonas, Aeromonas, Enterobacteriaceae, Yersinia, Staphylococcus, Streptococcus, Micrococcus, Acinetobacter, Moraxella, Vibrio, Escherichia coli.*

To obtain carcasses with a very good hygienic quality, the main aim during the slaughtering process is to have a very low initial microbial load (Dan, 2017). In the case of swine carcasses, the initial microflora differs from bovine carcasses, because of the different technological flow steps, like the scalding, depilation, and singeing.

The carcass spoilage is influenced by the following factors: a high initial load of psychrotrophs, increased temperature in the chillers, high aw (water activity) values on the surface of the carcasses close to 1.0. As a result, Gram negative psychrotrophs bacteria will represent the main microflora with the highest spoilage potential for refrigerated carcasses. If the meat is kept at temperatures below 7°C or lower, under aerobic conditions, *Pseudomonas,*

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Moraxella, Acinetobacter and *Psychrobacter* members will have the highest growth rate, hence their increased spoilage potential. Also, *Shewanella spp*. and some members of the *Entorobacteriaceae* family were able to grow and to produce spoilage metabolites.

MATERIAL AND METHOD

The research material was represented by 288 swine meat samples, collected between January and December 2021, from two pig slaughtering units in Cluj County. The samples were collected using a destructive method, before (warm carcass), and after chilling (24 hours), each month three samples from both the surface and the depth, in compliance with the current legislation (Reg. CE 2073/2005). From the surface of the carcasses, four slices, with a thickness of 2-3 mm were collected from different anatomical regions: the inner side of the thigh, chest, hind legs, and pelvic cavity. Samples were collected randomly, considering that they should come from carcasses obtained both at the beginning and at the end of the slaughtering process.

To determine the microbial load and configuration in broilers, 72 representative samples (poultry carcasses) were taken, from 8 batches, as follows: 9 carcasses/month, respectively batch, packed in a cryovac system, harvested between April and December 2021 at a slaughterhouse-distribution unit in the Transylvania region. In the slaughtering unit studied, the slaughtering process is automated, using modern equipment, with a production capacity of 10,000 birds / 8 hours. The microbiological examinations were carried out in the laboratory of the Food Inspection and Control discipline of the Faculty of Veterinary Medicine Cluj-Napoca.

A quantitative approach based on microbiological determinations analyses were carried out: psychrotrophic plate count, isolation of Pseudomonas, Aeromonas, Yersinia and Enterobacteriaceae, using selective media as follows: for aerobic plate count PCA agar (Merck), for Aeromonas and Pseudomonas, GSP agar (Merck), for Yesinia, CIN agar (Merck), and for Enterobacteriaceae – VRBD agar (Merck). Serial decimal dilutions (10-6) were obtained from 10 grams of meat and 90 ml water buffered peptone. The spreading method was used to inoculate 0.1ml onto the surface of two Petri plates. Incubation was realized at 20°C, for 72 hours. The biochemical confirmation test was realized using API 20 E and API 20 NE (Biomerieux). Statistical analysis was carried out using Origin 8.5 software by comparison of means by analysis of variance through ANOVA test or API LabPlus. The statistical interpretation of the results was realized according to the probability indicator: $p \leq 0.05$ (confidence level 95%). The results were depicted as log CFU/cm^2.

RESULTS AND DISCUSSIONS

From the analysis of the results regarding microflora of warm swine carcasses, we can say that if the slaughtering process is carried out in strict compliance with the hygiene practices, the microbial load presented lower values, not exceeding the maximum limits allowed or recommended by the current legislation, respectively the literature (Liora, 2013; Reg CE 2073/2005, Dan, 2017).

In swine carcasses case, the trimestral average of the total psychrotrophic count at the surface of warm carcasses presented different values, ranged between 3.80±0.60 log CFU/cm2 in trim. I and 5.01±0.30 log CFU/cm2 in trim. III, with a minimum of 2.63±0.44 log CFU/cm2 in January and a maximum of 5.22±0.26 log CFU/cm2 in September.

The microbial population at the surface of swine carcasses before chilling is depicted in Figure 1, being represented by the following genera: *Staphylococcus, Micrococcus, Lactobacillus, Neisseria, Aeromonas, Acinetobacter, Moraxella, Pseudomonas, Yersinia, Serratia, Hafnia, Proteus* and *Escherichia*.

In the case of the samples collected from the surface of refrigerated carcasses, we found that the dominant species is the Gram negative one, respectively 73.98%, and the Gram-positives are only 26.02%.

Among the Gram-negative bacteria, it was found that the psychrotrophic are the dominant population: *Pseudomonas* (23.93%), *Acinetobacter* (9.4%), *Moraxella* (7.69%), *Yersinia* (7.26%), *Serratia* (6.35%), *Hafnia* (4.27%), *Aeromonas* (3.84%), *Shewanella* (3.42%), *Escherichia* (2.99%), *Proteus* (1.79%) and *Enterobacter* (2.14%) (Figure 2).

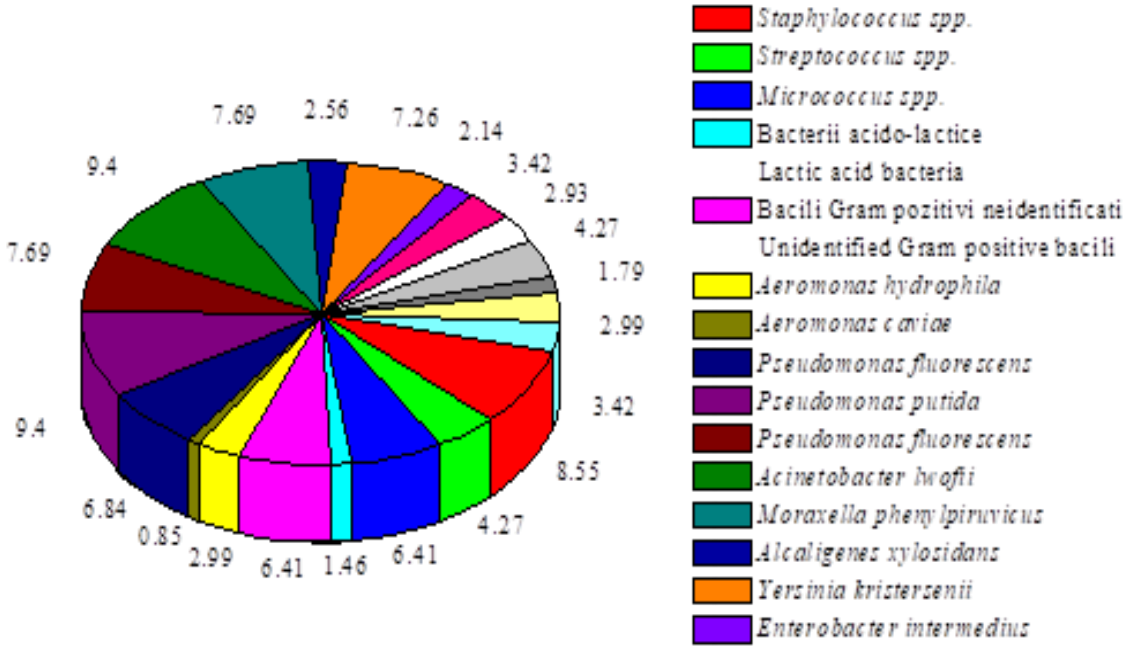

Figure 1. Microbial population at the surface of warm swine carcasses in slaughterhouse

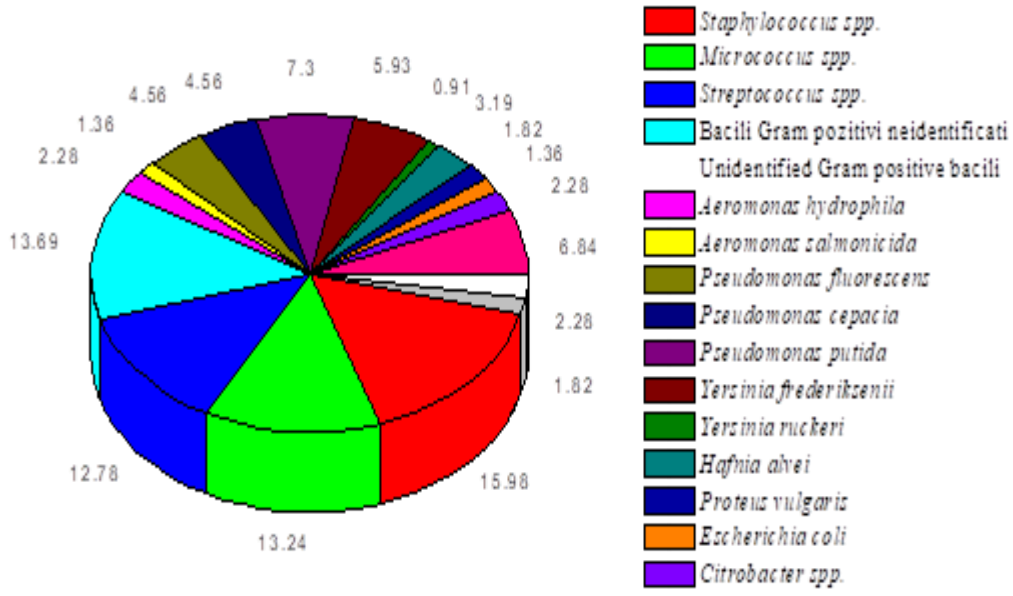

Figure 2. Microbial population at the surface of chilled swine carcasses in slaughterhouse

In the case of broiler carcasses, to identify the psychrotrophic bacterial species developed on culture media (SI, GSP-agar, Yersinia-agar, VRBD-agar), API 20NE and API 20E (Biomerieux) biochemical confirmation tests were performed. After incubation of the API galleries at the thermostat, differentiated according to the type of test used, the results were interpreted with the help of the identification software.

For the rest of the results, additional tests were performed, different depending on the product provided by the computer program. When the microorganisms could not be identified even with the help of these tests, the result was classified as an "unidentified bacterial species".

The main bacterial species identified and their incidence in the case of broiler chicken carcasses are shown in the graph in fig. 3.

From the processing of the results presented in the graph in figure 3, it follows that the predominant bacterial species are Gram negative, respectively 70.26%, while Gram positive bacteria represented 29.44% of the total.

Among Gram positive bacteria, 7.01% are represented by staphylococci, 3.44% by streptococci, 8.77% by micrococci and 10.52% by lactic acid bacteria. The differentiation between the coccoid formations was made based on the bacterioscopic examination, the catalase and oxidase test.

Among the representatives of the *Enterobacteriaceae* family, the following species were identified, based on API 20 E tests: *E. coli* (5.26%), *Vibrio vulnificus* (1.69%), *Citrobacter freundii* (3.38%). Among these species, health problems can be caused by *Vibrio parahaemolyticus* and *E. coli*, which have pathogenic strains for humans.

The rest of the species are part of the category of psychrotrophic germs of the *Enterobacteriaceae* family, frequently involved in triggering the alterative processes of meat at loads between 106 - 107 cfu/cm2 (Gram L. et al, 1999).

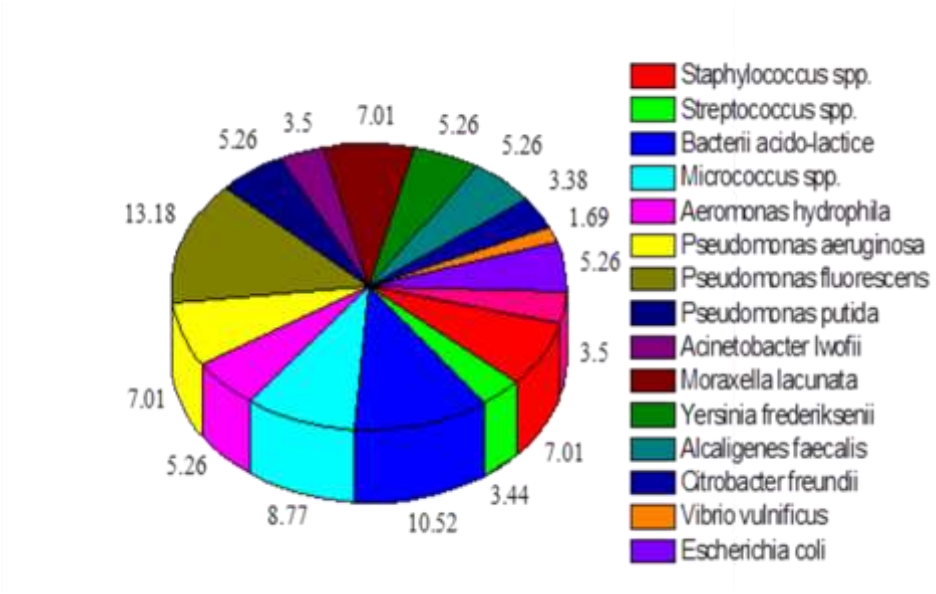

Figure3. Incidence of bacterial species at the surface of poultry carcasses.

Regarding the germs of the *Yersinia* genus, from the results obtained, no pathogenic species were identified, but only *Yersinia frederiksenii* (5.26%)

Among the Gram-negative bacteria, it was found that psychrotrophic bacteria predominate, of which: the genus *Pseudomonas*, with 25.46%, the genus *Moraxella* with 7.01%, the genus *Aeromonas* with the species *Aeromonas hydrophila* 5.26%.

CONCLUSIONS

Microbial load from the surface of carcasses is significantly influenced by the temperature in the chilling room of the slaughterhouse, if the temperature is inadequate, the microbial load is significantly higher. The microbiological assessment carried out on pork carcasses demonstrates the role of psychrotrophic microorganisms in the spoilage processes in case of improper monitoring of the slaughtering processing.

As broiler carcasses are stored for longer periods of time in refrigeration spaces, the bacterial load on the surface shows an upward evolution, reaching values of up to 8.0-9.0 log cfu/cm2, the level of growth of the bacterial population, such as and the configuration of the germs present on the surface of the carcasses being dependent on the refrigeration temperature and the relative humidity of the storage space. Based on these aspects, we can appreciate that the psychrotrophic spoilage microorganisms are (also) important from an economic point of view, if the microbial population exceeds the level of 6.0 log cfu/cm2,

because of the production of sensory changes, respectively of the reduction of the limit duration for consumption.

ACKNOWLEGMENTS

The microbiological examinations were carried out in the laboratory of the Food Inspection and Control discipline of the Faculty of Veterinary Medicine Cluj-Napoca. The data comes from different studies.

REFERENCES

ADRIAN-COSMIN, C.A.R.A.I.M.A.N., SORIN, D. and SIMON, P., 2023. GREEN BUILDINGS AND THEIR BENEFITS IN THE CONTEXT OF SUSTAINABLE DEVELOPMENT. *Annals of'Constantin Brancusi'University of Targu-Jiu. Economy Series*, (1).

Alban, L., Antunović, B., Belous, M., Bonardi, S., García-Gimeno, R.M., Jenson, I., Kautto, A.H., Majewski, M., Oorburg, D., Sakaridis, I. and Sirbu, A., 2023. Mapping ways of detecting and handling antimicrobial residues in pigs and pig meat in-and outside Europe. *Food Control*, p.109899.

Belous, M., Mihaiu, M., Tabaran, A., Reget, O.L., Cîmpean, R. and Dan, S.D., 2023. Assessment of the spoilage microflora in swine carcasses. *Safe Pork*, *13*(1).

Dan, S.D., Mihaiu, M., Reget, O., Oltean, D. and Tăbăran, A., 2017. Influence on week organic acids on pathogens on swine carcasses.

Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I. and Nielsen, T.F., 1999. Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. *Applied and environmental microbiology*, *65*(3), pp.969-973.

Liora, M.I.H.A.I.U., Mihaiu, M., TĂBĂRAN, A., DAN, S.D., CORDIŞ, I.V., Pivariu, B. and Cordea, D., 2013. Antimicrobial Resistance Evaluation of Pathogen Salmonella Strains Isolated in Pork and Poultry Meat. *Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine*, *70*(2).

Regulation (EC) 2073/2005, date access October, 2023