Assessment of the spoilage microflora in swine carcasses

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Abstract
The aim of our study was to perform a microbial risk assessment at warm and chilled swine carcasses represented by the psychrotrophic bacteria. The research material was represented by 288 swine meat samples, collected between January and December 2021, from two pig slaughtering units in Cluj County. 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. 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/cm^2 in trim. I and 5.01±0.30 log CFU/cm^2 in trim. III. *Pseudomonas* spp. count in unit A ranged between 2.58 ±0.48 log CFU/cm^2 in trim. II and 3.46±0.73 log CFU/cm^2 in trim. I. Initial microflora from the surface of swine carcasses in both slaughterhouses was dominated by Gram positive bacteria (55.69% in the case of slaughterhouse A, respectively 56.36% in the slaughterhouse B). 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.* 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.

Introduction
The initial microbial load and configuration of the microflora at the surface of carcasses is influenced by the animal's health before the slaughter process, the time and welfare conditions during transport, and with a greater extent by the implementation of the GHP (Good hygiene practices) and GMP (Good manufacturing practices) during the slaughtering process by all the workers (Nychas, 2000; Napravnikova, 2002; Dan, 2005). In order 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, 2007). In the case of swine carcasses, the initial microflora differs from bovine carcasses, as a result of the different technological flow steps, like the scalding, depilation, and singeing. As a result, the surface microbial load is quite low, below 10^3 or even 10^2 CFU/cm^2 (Gill and Bryant, 1992; Gill *et al*., 1997). However, after the subsequent slaughtering steps, like evisceration, splitting and chilling, a recontamination of swine carcasses may occur, with a similar microbial population isolated in the case of bovine carcasses (Brown, 1982; Davies and Board, 1998; Jackson *et al*., 1997, Brown, 2000). A major source of contamination of the carcasses is represented by evisceration. Non-compliance with the hygiene rules during this step, not sealing the anus, or puncturing the viscera, leads to the massive contamination of the meat (Nesbakken, 2000).The rapid cooling of the carcasses at low temperatures, the increased speed of the air currents, can lead to a reduction of the bacterial population, while a slow chilling will allow the growth of psychrotrophs, that will become the dominant population, being able to spoil the meat in a short time (Day, 2000; Hansen and Bautista, 2000). The carcass spoilage is influenced by the following factors: a high initial load of psychrotrophs, increased temperature in the chillers, high a_w (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, Moraxella, Acinetobacter* and *Psychrobacter* members will have the highest growth rate, hence their increased spoilage potential. Also, S*hewanella* spp. and some members of the Entorobacteriaceae family were able to grow and to produce spoilage metabolites. In our study we tried to carry out am microbial risk assessment at swine carcasses represented by the psychrotrophic bacteria, from two slaughtering units in Cluj County, Romania,

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having as specific objectives: the assessment of the microbiological load and configuration at the level of swine carcasses before and after the chilling.

Materials and Methods

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 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. It was also considered that the collection that reflect as accurately as possible the level of contamination and the microbiological configuration of the pig carcasses. The samples, collected under sterile conditions, were placed in sterile polyethylene bags, being transported to the laboratory of the Food inspection, located in 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. 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 discussion

In the case of slaugherhouse A, 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/ cm^2 in trim. I and 5.01±0.30 log CFU/cm^2 in trim. III, with a minimum of 2.63±0.44 log CFU/cm^2 in January and a maximum of 5.22±0.26 log CFU/cm^2 in September. Studies conducted by Zweifel *et al.* (2005) in five commercial slaughterhouses (Switzerland), highlighted a total psychrotrophic count ranged between 2.18 log CFU/cm^2 and 3.70 log CFU/cm^2, lower when compared with our results, which demonstrate the importance of the hygiene practices in these units. Pinto *et al.,* (2004), reported relatively low psychrotrophic count, ranged between 3.05 log CFU/cm^2 and 4.0 log CFU/cm^2. Regarding the load of germs from *Pseudomonas* spp. in unit A, the average ranged between 2.58±0.48 log CFU/cm^2 in trim. II and 3.46±0.73 log CFU/cm^2 in trim. I, with a minimum of 2.23±0.15 log CFU/cm^2 and a maximum of 4.76±0.66 log CFU/cm^2, both values recorded in January. In the case of total psychrotrophic count in the slaughterhouse B, the results were lower, but no significant differences were calculated ($p>0.05$). *Pseudomonas* spp. was identified in all collected samples, demonstrating that they are also the dominant population in the case of warm swine carcasses (Gill, 1996; Buys *et al*., 2000). Similar results were reported by Nel *et al*., 2004, that revealed 3.96 log CFU/cm^2 for the *Pseudomonas* spp. Microbial load and configuration of the pseudomonads isolated at the carcass level are largely dependent on the microflora in slaughterhouse environment, and the results can have differences from one slaughterhouse to another (Lowrie, 1998). *Aeromonas* spp. were isolated only in nine samples in the case of unit A (25%), the average microbial load ranged between 2.66±0.37 log CFU/ cm^2 in trim. III and 4.0±0.47 log CFU/cm^2 in trim. I, with a minimum of 2.4 log CFU/cm^2 in August and a maximum 4.7 log CFU/cm^2 in March. The average microbial load of *Yersinia* spp. in the case of the samples from the surfaces in unit A presented low values, between 2.80 ±0.23 log CFU/cm^2 in trim. I and 3.47±0.3 log CFU/cm^2 in trim. III, with a minimum of 2.5 log CFU/cm^2 in July, and a maximum of 3.7 log CFU/cm^2 in February. The microbial load of *Enterobacteriaceae* ranged between 1.97±0.06 log CFU/cm^2 in trim. I and 3.48±0.25 log CFU/ cm^2 in trim. III, with a minimum of 1.63±0.15 log CFU/cm^2 in May and a maximum of 3.73±0.50 log CFU/cm^2 in August. The maximum admitted level by the Reg CE 2073/2005, of 2.5 log CFU/cm^2, exceeded in the

case of 11 samples collected in trim. II and III respectively in 30.55% of the total samples. The results obtained by Nel *et al.* (2004), and Mayr *et al*. (2003) revealed higher levels of Enterobacteriaceae load, on the surface of swine carcasses, of 4.15 log CFU/cm^2, respectively 3.56 log CFU/cm^2, when compared with our study. Initial microflora from the surface of swine carcasses in both slaughterhouses was dominated by Gram positive bacteria (55.69% in the case of slaughterhouse A, respectively 56.36% in the slaughterhouse B). 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.*

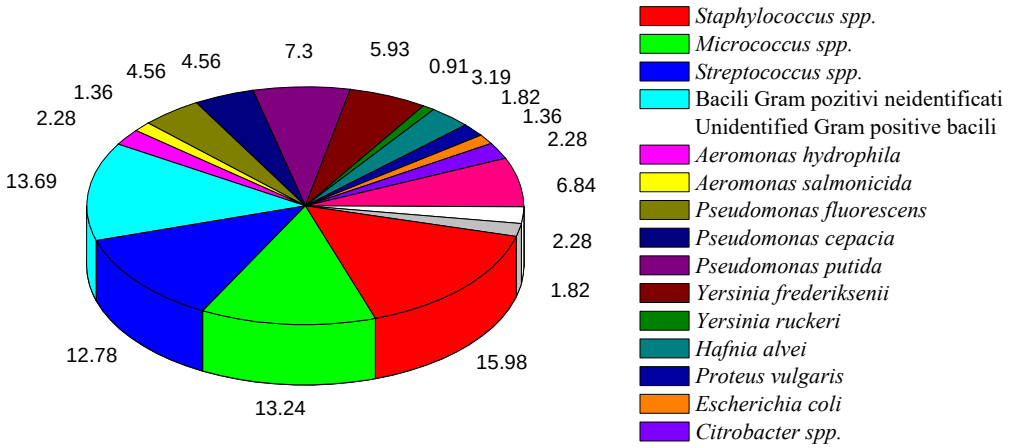

Figure 1. Microbial population at the surface of warm swine carcasses in slaughterhouse A

Fig. 48 Incidenta speciilor bacteriene la suprafaţa carcaselor calde de porcine din unitatea A

The incidence of bacterial species at the surface of warm pork carcasses from a slaughterhouse

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 (Cousin, 2000; Reg CE 2073/2005, Dan, 2007). In the case of chilled swine carcasses, the total psychrotrophic count presented different values during the Ist and the IVth quarter, associated with the temperatures in the chilling rooms of the slaughterhouses A and B. Total psychrotrophic count of the samples from unit A ranged between 3.86±0.59 log CFU/cm^2 in trim. I and 5.16±0.30 log CFU/cm^2 in trim. III, with a minimum value of 3.33±0.20 log CFU/cm^2 in January and 5.46±0.23 log CFU/cm^2 in July (fig. 51). Out of all the examined samples, eight samples exceeded the maximum level of 5.0 log CFU/cm^2 (22.22%). Regarding unit B, it was found that the average psychrotrophic count was higher than in the case of unit A ($p<0.05$), which demonstrated worse hygiene practices. Microbial load ranged between 5.64±0.23 log CFU/cm^2 in trim. I and 5.86±0.15 log CFU/cm^2 in trim. III, with minimum values of 5.45±0.22 log CFU/cm^2 in February and 6.46±0.28 log CFU/cm^2 in July. Lower microbial load was reported by Pinto (2004), ranged between 2.5 - 3.82±0.21 log CFU/cm^2. Rho *et al*. (2001), established after 24 hours of refrigeration, the total psychrotrophic count was 3.0 log CFU/cm^2, significantly lower when compared with our study, which emphasize the importance of applying the hygiene practices during the entire slaughtering process. *Pseudomonas* count in the case of the samples collected from slaughterhouse A ranged between 3.12±0.48 log CFU/cm^2 in the Ist quarter and 5.11±0.97 log CFU/cm^2 in the IIIrd one. For the samples taken from unit B, the average level of contamination with *Pseudomonas* spp. ranged between 3.77±0.48 log CFU/cm2 in the Ist quarter and 5.6±0.61 log CFU/cm^2 in the IIIrd one, with a minimum value of 3.1±0.15 log CFU/cm^2 in January and a maximum of 6.06±0.15 log CFU/cm^2 in July. The level of contamination in the two units did not present significant differences ($p\geq0.05$) being variable, depending on the microclimate conditions in the slaughterhouse, as well as on compliance with hygiene practices. Li *et al.* (2006), reported lower pseudomonads load levels in comparison with our study, of 2.65 log CFU/cm^2. Also, Mayr *et al.* (2003), reported 3.91±0.14 log CFU/cm^2 for pseudomonads after 24 hours after slaughter.

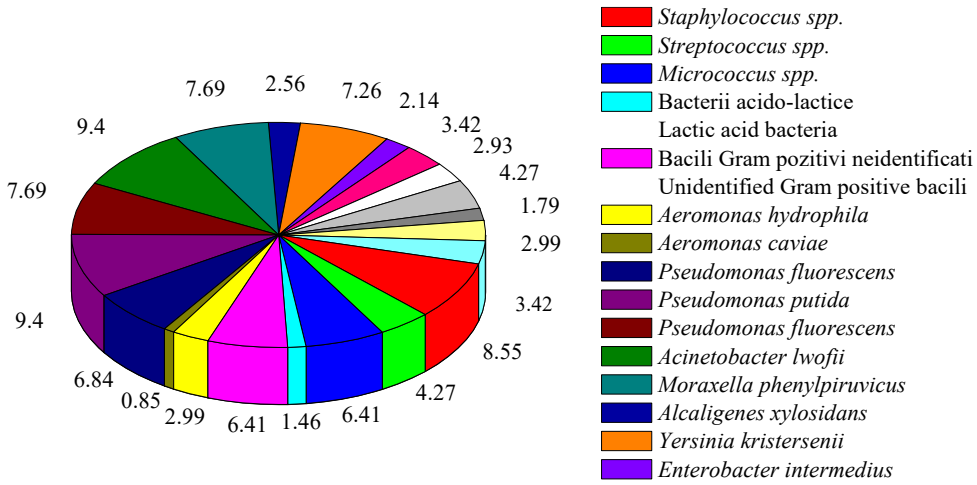

Figure 2. Microbial population at the surface of chilled swine carcasses in slaughterhouse A

The incidence of bacterial species at the surface of refrigerated pork carcasses from A and B slaughterhouse

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). These results are consistent with other studies that revealed that the germs of the genus *Pseudomonas* have the highest microbial loud, up to 40% in the first 48-72 hours after the slaughter (Lacroix *et al.,* 2000; Ellis and Goodacre, 2001; Mayr *et al.,* 2003; Li *et al*., 2006).

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.