

INCIDENCE OF CUTANEOUS *Staphylococcus* species IN EXTENSIVELY RAISED SWINE

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Abstract

The commensal/opportunistic bacteriome in various animal species includes potentially pathogenic *S. aureus*. Widespread in humans, on the skin or mucosae, it induces a highly variable pathology, depending on its toxins and virulence, on the host and also on the environment. Much less is known about other staphylococci and their involvement in swine pathology. This research envisaged the incidence of *Staphylococcus* spp., in samples collected from extensively raised clinically healthy pigs, of the same age, during the period 2019-2020. The samples (n=49) were provided from farms of different sizes and in different years. Cotton swabs were used to sample secretions from anterior nares of individual pigs, and from the skin behind the ears of the animals. Classical microbiology methods were used to isolate *Staphylococcus* spp. and the isolates were identified using biochemical tests (API Staph, BioMerieux). Forty species of *Staphylococcus* were identified: *Staphylococcus xylosus* (47,5%), *Staphylococcus lentus* (30%), and *Staphylococcus sciuri* (22,5%). These bacteria seemed often present as a commensal animal-associated bacteria, but in some cases they could become pathologic in some diseases like bovine mastitis, and exudative epidermitis.

Key words: swine, extensive raising, skin, staphylococci, pathogenicity.

INTRODUCTION

MRSA represents lately an increasing problem in humans as well as in livestock. Nevertheless, the bacterial co-colonization of the skin in MRSA carriers has been poorly investigated (Strube et al., 2018).

The genus *Staphylococcus* currently comprises 81 species and subspecies, and most members of the genus are mammalian commensals or opportunistic pathogens that colonize niches such as skin, nares, and diverse mucosal membranes (Haag, et. Al 2019).

The distribution of the normal flora of *Staphylococcus* spp. is an important factor to understand the epidemiology of skin diseases in humans and animals (Nagase et al., 2001).

Despite the abundance of literature characterizing staphylococcal pathogenesis in humans, *S. aureus*, a major cause of infection and disease in a plethora of animal hosts, leading to a significant impact on public health

and agriculture. Infections in animals are deleterious to animal health, and animals can act as a reservoir for staphylococcal transmission to humans. While about 20 to 30% of the human population carries *S. aureus*, the prevalence of the bacteria varies from host to host in animal species, the percentage of carriers reaching 90% in chickens, 42% in pigs, 29% in sheep, and between 14 and 35% in cows and heifers ((Haag, et. Al 2019).

Methicillin-resistant *S. aureus* (MRSA) emerged by the integration of resistance mechanisms in methicillin-susceptible *S. aureus* (MSSA). The acquisition of *mecA* or *mecC* is a public health concern due to limited options for treatment. Moreover, MRSA infections are related to longer hospitalization periods and higher mortality (Porerro et al., 2014).

Staphylococcus sciuri is an important pathogen for humans, because it is responsible for endocarditis, peritonitis, septic shock, urinary tract infection, pelvic inflammatory disease,

and wound infection. However, little information is known regarding the pathogenicity of *Staphylococcus sciuri* in animals (Chen et al., 2007).

However, those species have been isolated also from infections, both in veterinary and human medicine. More investigation into the role of the *S. sciuri* species group as commensal and pathogenic bacteria is required to fully assess its medical and veterinary importance (Nemeghaire et al., 2014).

Staphylococcus xylosus, *Staphylococcus lentus*, *Staphylococcus equorum*, *Enterococcus faecalis*, and *Pantoea agglomerans* were identified as pathogens in bovine mastitis (Da-Cheng Hao, 2018).

MATERIALS AND METHODS

Sampling. The samples were collected during the period 2019-2020, from extensively raised clinically healthy pigs, of the same age. The samples (n = 49) were provided from farms (n = 5) of different sizes and in different years. Cotton swabs were used to sample secretions from anterior nares of individual pigs (n = 34), and from the skin behind the ears of the animals (n = 15).

The envisaged farms were non-professional exploitations, with fattening pigs which are subject to commercial activities, namely, the sale of live pigs and meat in Romania, and were classified as biosecurity level II or III. The biosecurity level II included the general biosecurity levels: introduction to the exploitation register provided by the by the Order of the President of the National Sanitary Veterinary and Food Safety Authority no. 40/2010 on the approval of the Veterinary Sanitary Norm for the implementation of the process of identification and registration of pigs; keeping pigs in fenced areas, without the possibility of coming into contact with domestic pigs from other holdings or wild boars; prohibition of access to exploitation of foreign persons and the use of brief protective equipment, namely overalls or a work gown (<https://www.meat-milk.ro/ansvsa-proiect-privind-regulile-de-biosecuritate-exploatatiile-de-porcine/>).

Sampling procedures. The samples were directly inoculated into simple broth

supplemented with serum, and were incubated in aerobic conditions for 24 h at 37°C. After mixing gently the broth, sub-cultures from the broth were performed by streaking one loop of 10 µl over the surface of the Chapman agar and incubating the plate at 37°C for 24 h in aerobic conditions.

Based on the morphology of the staphylococcus colonies on Chapman agar (yellow colonies) (Figure 1), they were individually subcultured on the Columbia Agar with 5% Sheep Blood agar, and were incubated at 37°C for 24 h.

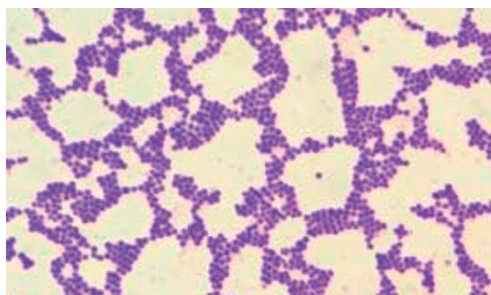


Figure 1. Coccoid bacteria - Gram-stain, x 100

Once the identity of the genus *Staphylococcus* was confirmed and to avoid the contamination, a colony from the subculture was picked and streaked on a new plate on simple agar, and incubated 37°C for 24 h. These re-sub-cultured bacterial isolates were stored in 40% glycerol at -20°C.

Staphylococcus spp. were then identified based on the colony characteristics, morphology, Gram stain, and colony pigmentation. Further, the identification procedure was continued by use of biochemical tests (API Staph, BioMerieux) (Figure 2).

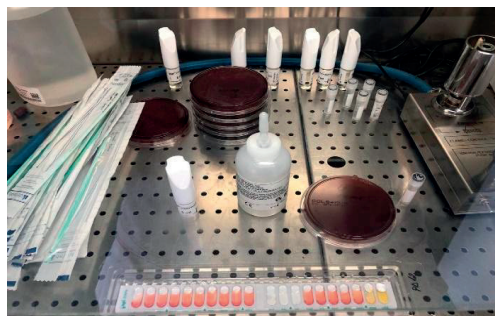


Figure 2. Api Staph kit (BioMerieux)

RESULTS AND DISCUSSIONS

The dominant species were *Staphylococcus xylosus* (47.5%), *Staphylococcus lentus* (30%), and *Staphylococcus sciuri* (22.5%).

The rates of detection of the *Staphylococcus* spp. on the skin samples, from the five different farms are shown in the Table 1. *Staphylococcus* spp., were detected on the skin of pigs 81.63%.

Table 1. Farm based positivity for *Staphylococcus* from the skin of the experimental pigs

Sampling	No. of samples	No. of positive (%)
A Farm	5	3 (60%)
B Farm	5	5 (100%)
C Farm	7	6 (85.71%)
D Farm	14	12 (85.71%)
E Farm	18	14 (77.77%)

The distribution of the *Staphylococcus* spp. according to the sampling area (the anterior nares of individual pigs, or from the skin behind the ears) are shown in Table 2.

The dominant species of *Staphylococcus* spp., were mostly similar in both sampling locations, except *Staphylococcus sciuri*, which was more frequently isolated from the anterior nares (23.52%), compared with skin behind the ears (6.66%). Similarly, *Staphylococcus xylosus* was found in a higher percentage (53.33%), on the skin behind the ears.

Table 2. The predominant species of *Staphylococcus* according the sampling area

	No. of positive from anterior nares = 34 (%)	No. of positive from skin behind the ears = 15 (%)
<i>S. xylosus</i>	11 (32.35 %)	8 (53.33%)
<i>S. lentus</i>	8 (23.52 %)	4 (26.66%)
<i>S. sciuri</i>	8 (23.52%)	1 (6.66%)

The prevalence of *Staphylococcus* spp. was different depending on the farm where the bacteria was isolated, as indicated in Figure 3. Knowing that the five farms were located in different areas, and data shown us that two species of the three isolated are dominant species represented by *Staphylococcus xylosus* (Farm B = 60%, Farm D = 57.14%, and farm E = 38.88%) followed by *Staphylococcus sciuri*

(Farm A = 66.66%, and Farm C = 57.14%), and *Staphylococcus lentus* (Farm C, and D = 28.57%, Farm E = 27.77%, and Farm B = 20%).

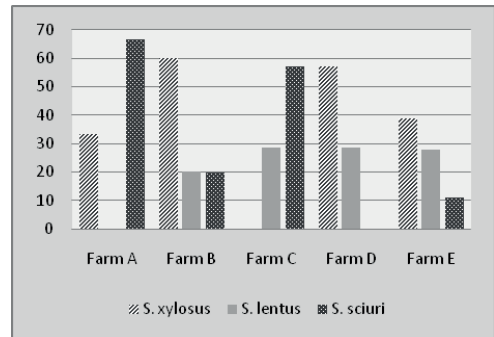


Figure 3. The dominant species of *Staphylococcus* isolated by the farm

Staphylococcus xylosus was isolated most frequently from the skin behind the ears (53.33%) and only 32.35% of isolated from anterior nares, *Staphylococcus lentus* was isolated in approximately equal percentages from the anterior nares (23.52%), and the skin behind the ears (26.66%), and the last one, *Staphylococcus sciuri* was mostly isolates from the anterior nares (23.52%). These results suggest that *Staphylococcus xylosus* and *Staphylococcus lentus* were well-adapted to the skin environment of healthy pigs.

Staphylococcus xylosus is a commensal of the skin of animals and humans. This last species was virtually defined as a non-pathogenic staphylococcus, but a few strains of *S. xylosus* were related to animal and human opportunistic infections, and murine dermatitis (Frisoni et al., 2007), as well as the most frequently isolated species associated with cow mastitis (Kot et al., 2012).

Currently little information is available regarding the pathogenicity of *S. sciuri* in animals (Chen et al., 2007). *S. sciuri* is mostly recovered from skin and mucous membranes of animals and has long been considered a non-pathogenic commensal bacterium. During the last decade this has been associated with several cases of bovine mastitis, as well as with goats infected with peste des petite ruminants virus, and also from cases of canine dermatitis, as well as from several outbreaks of fatal

exudative dermatitis in piglets (Beims et al, 2016).

S. sciuri group has also been found to carry multiple virulence and resistance genes. Indeed, genes involved in biofilm formation or coding for toxins responsible for toxic shock syndrome and multi-resistance, similar to those carried by *Staphylococcus aureus*, were detected (Nemeghaire et al., 2014). The presence of resistance and virulence genes similar to those found in *S. aureus* enhances the hypothesis that *S. sciuri* might be an important reservoir for these genes (Nemeghaire et al., 2014) for more pathogenic *Staphylococcus* species (Harrison et al., 2014).

CONCLUSIONS

The present study was conducted to reveal the incidence of cutaneous *Staphylococcus* species in extensively raised healthy swine. The high isolation rate of *Staphylococcus* spp. And the presence of several species: *Staphylococcus xylosus*, *Staphylococcus lentus* and *Staphylococcus sciuri* indicated an important pathogenic potential of the bacteriome in healthy animals.

These bacteria, although seemed often present as commensal animal-associated bacteria, in frequent and close contact between species on the farms may jump over species and cause pathologies bovine (mastitis), other pigs (exudative dermatitis), and could also preserve the reservoir of antibiotic resistance genes.

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