STAPHYLOCOCCUS SPECIES IN DIFFERENT AGE GROUPS OF PIGS IN LATVIA

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Abstract
Antibiotic resistance of *Staphylococcus* is increasing worldwide. New antibiotics are used in big amounts in the meat production more and more. As a zoonosis, *Staphylococcus aureus (S. aureus)* is found in various species of animals and people, especially in those, who are working on commercial swine farms and slaughterhouses. The aim of the study was to find out the occurrence of *S. aure*us in different age groups of pigs on commercial swine farms in Latvia. Microbiological samples (n=702) including nasal, rectal, milk and air samples were collected during October 2013 from three closed farms of different sizes and several age groups of pigs and investigated with microbiological standard methods. *S. aureus* was found in all swine farms. The occurrence of *S. aureus* in Latvian pig farms was 41% and the highest occurrence of *S. aureus* was among 3-3.5 month old piglets. *S. aureus* was 1.8 times more frequently found in nasal than in rectal samples, and only in 28.38% of pigs *S. aureus* was in both - nasal and rectal samples. *S. aureus* was found also in sow milk in 13% of samples and in 3 samples of air (n=23).
Key words: *staphylococcus, S. aureus,* pig farms.

Introduction

*Staphylococcus aureu*s is an opportunistic pathogenic Gram positive bacteria and the causative agent of a wide panel of infections ranging from superficial lesions to life-threatening septicaemia (Charlier et al., 2009; Boucher et al., 2010). It is no longer a human healthcare-associated problem, but is now a community-associated problem (Morgan, 2008). Methicillin resistant *Staphylococcus aureus* (MRSA) were found to colonize and infect various animal species including horses, cows, dogs and cats, rabbits and other companion animals. A special focus has been put on the isolation of MRSA from pigs in several countries including the Netherlands, Denmark, France, Canada, the USA and Singapore. However, in the majority of reports pigs were colonized, but were not infected by MRSA (Kock et al., 2009). According to the data of European Food Safety Authority (EFSA), in 2009 MRSA CC398 (MRSA genome type that is common in animals and people) was found in 39% percent of pigs in Dutch slaughterhouses and in 13% of fattening pigs in Germany, and one of the most frequent hosts of this MRSA CC398 type are pigs.

Currently, *S. aureus* occurrence and antibiotic resistance in the world increases and with it also the concern about *Staphylococcus aureus* and MRSA colonization in swine farms in Europe and spreading from animal to human. Besides all European country data about the prevalence of *S. aureus* in pigs, no investigations have been made in the situation of the Latvian pork industry. Therefore, the aim of the study was to find out the occurrence of *S. aureus* in commercial swine farms in Latvia.

Materials and Methods

The collection of microbial samples took place in October 2013 in three pig farms from three different regions of Latvia – Kurzeme, Vidzeme and Riga. The description of each pig farm is given in Table 1. The body condition of swine was scored according to Stockmanship standards (Carr, 1998). Evaluation of animal welfare, hygiene, and microclimate conditions in the pig farms was based on Council Directive 2008/120/EC of 18 December 2008, laying down minimum standards for the protection of pigs and microclimate standards according to M. Muirhead suggestions (Muirhead et al., 2013).

Sampling

Pigs were divided into four groups: pre-weaned piglets with sows, 3-3.5 months old piglets, 4-4.5 months old piglets and fattening pigs (shortly before slaughter) (see Table 2). Nasal and rectal samples were collected from each group. Also, from each farm milk samples (n=69) and air samples (n=23) were collected. In total, 305 pigs and 702 microbiological samples were investigated. Nasal and rectal samples were collected with sterile transport swabs (Meus, IT). Milk samples were collected in 50 mL amount sterile tubes without preservative. Air samples were collected using Baird-Parker Agar plates according to Koch's sedimentation method (Boucher et al., 2010). All microbiological samples were stored at +4 °C and the first isolation was made during 24 hours after the sample collection.

Microbiological examination

The research was performed at the Institute of Food and Environmental Hygiene of Latvia University of Agriculture Faculty of Veterinary Medicine. Samples from transport swabs were transferred on Baird-Parker Agar with egg yolk supplement (Becton, Dickinson, USA), and incubated at 37 °C for 24 hours according to LVS EN ISO 6888-1:1999 A1:2003 'Microbiology

Table 1

Characterisation of pig farms

Pig farm	Number of sows	Number of fattening pigs	Health conditions	Hygiene and welfare conditions	Antibiotic usage
A	250	1500	Somewhat thin (score 2.5) and thin sows (score 2), reduced fertility and birth rate, cannibalism	Some dirty cages and pens, slatted floors, no available straw and environment enrichment	For treatment
B	1200	8000	Good health and condition (score 3.5)	Dirty and wet pens of fattening pig groups, slatted floors, concrete solid floor with straw in 4-4.5 month old group.	For treatment and prophylaxis
C	20000	12000	Scars and purulent lesions on joints and phalanx, weak and thin (score 2) piglets, sows in normal condition (score 3), cannibalism.	All pens dirty and wet, no available straw and environment enrichment, too cold for piglets (24 °C in newborn piglet pens), slatted floors.	For treatment

Table 2

A number of investigated pigs, milk and air samples in each farm

Group of pigs/sample type	Number of investigated pigs/samples		
	Farm A	Farm B	Farm C
Pre-weaned piglets with sows	32	32	32
3-3.5 month old piglets	15	25	24
4-4.5 month old piglets	24	24	24
Fattening pigs	25	24	24
Milk	18	25	26
Air	6	9	8

S. aureus and other species - Part 1: Technique using Baird-Parker agar medium - Amendment 1: Inclusion of precision data'. After incubation, positive colonies were inoculated on Mannitol Salt Agar plates (Biolife, IT) at 37 °C for 24 hours. Rabbit Coagulase plasma (Becton Dickinson, USA) slide coagulase test was made with colonies from Mannitol Salt Agar plates. Coagulase positive colonies with positive reaction on MSA plates were determined as *S. aureus*-like and were inoculated on CHROMagar Staph aureus plate (Becton Dickinson, USA) at 37 °C for 24 hours. Isolates were confirmed to be *S. aureus* by examining previous tests. Samples were categorised positive, if at least one *S. aureus* positive colony-forming unit was isolated.

Results and Discussion

The Genus *Staphylococcus* consists of a variety of opportunistic pathogens of variable relevance in veterinary medicine. The most clinically relevant staphylococcus in veterinary medicine is the coagulase positive Staphylococcus aureus. A noted property of staphylococci is their ability to become resistant to antimicrobials (Weese and Duijkeren, 2009; Brown et al., 2005).

Staphylococcus aureus is an important cause of food poisoning, pneumonia, wound and nosocomial bacteremia. It is one of the natural components of microflora and may exist in environment, on skin and in mucus. Most animals may be colonized with *S. aureus*, but only recently MRSA strains were isolated from several food production animals, including pigs, cattle, chicken and other animals (Boucher et al., 2009; Weese and Duijkeren, 2009).

In our research, *S. aureus* was found in all three pig farms. Results showed that 41% of tested pigs were *S. aureus* positive. *S. aureus* was found in 34% of nasal samples and in 19% (Figure 1) of rectal samples; in addition, in 28.4% of pigs *S. aureus* was found in both – nasal and rectal samples, but in 19.8% of cases - only in nasal samples and in 5.9% of cases - in rectal samples.

Hypothetically our investigation is similar to Belgian MRSA research (Dewaele et al., 2013), because MRSA come from *S. aureus* species isolates and in Belgian research the highest sensitivity to

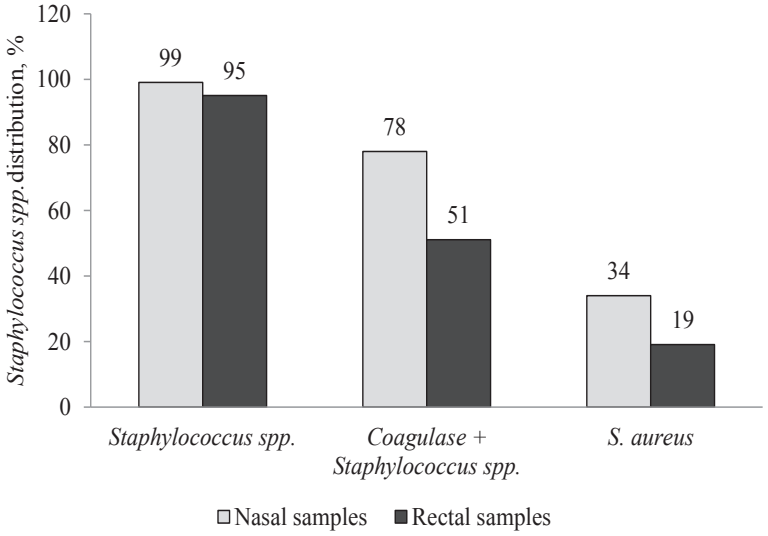

Figure 1. Comparison of *Staphylococcus spp.* distribution in nasal and rectal samples from pigs %.

determine MRSA carriage was found in the samples taken from nares 0.83, but from rectum - only 0.47. In I. Dewaele study, the best combination for sample taking is nares and perineum (sensitivity 0.96), nares and skin (sensitivity 0.92) and nares and rectum (sensitivity 0.89).

Housing conditions of fattening pigs have considerably changed in the past decades from extensive systems with large space allowance, substrate and/or outdoor housing, to intensive husbandry systems that have been developed for large scale production of pork. In these intensive husbandry systems, fattening pigs are housed with high housing density and without substrate. However, the intensive husbandry systems cause welfare problems for pigs. The main reason of these welfare problems is that the intensive housing conditions do not fulfil the internal need of the pig to perform species-typical behaviour. Pigs housed in barren environments show more abnormal agonistic behaviour, more manipulative social behaviour and have a higher level of aggression than pigs housed in pens with straw bedding, and it was concluded that these behaviours indicate welfare problems (Beattie et al., 1995; Jonge De et al., 1996). Stress caused by housing and management of pigs may not only affect animal welfare, but also the acceptance of the product by the consumer and the productivity. For example, stress caused by mixing of unfamiliar pigs reduced the growth rate for weeks (Ekkel, 1996).

This study has found that there is a difference between *S. aureus* occurrence in pig farms (see Figure 2). *Staphylococcus spp.* and coagulase positive

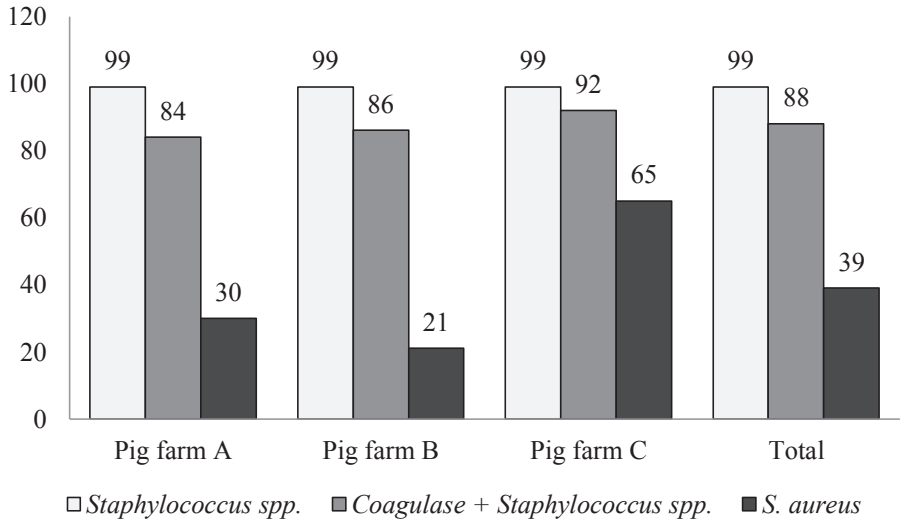

Figure 2. *Staphylococcus spp*. distribution in pig farms %.

staphylococcus as environmental microorganisms were highly spread in all three pig farms (in total, S*taphylococcus spp. w*ere found in 99% of nasal samples but in 78% of nasal samples – coagulase positive staphylococcus), but *S. aureus* was three times less spread in the pig Farm B. One of the reasons, that explains these rates, is antibiotic usage for prophylactic purposes in Farm B, but in Farms A and C - only for treatment. In addition, conditions of animal welfare and hygiene were best in Farm B. *S. aureus* occurrence was highest in the pig Farm C, where pig breeding, animal welfare and hygiene conditions were worst. In this farm, despite our country regulations and Council Directive 2008/120, sows were kept all the time in cages (during gestation, artificial insemination and gestation period) and in farrowing crates during piglet suckling period. There was no strain available for pigs in this farm. In addition, in pig Farm C pigs were more stressful than in Farm A and C and cannibalism signs were seen (bite wounds on ears, ducks, and neck).

Weaning and forming of new groups is a stressful time for all species of animals and can result in negative effects on the neonate after weaning. Stressful events such as weaning, forming of new groups and transportation can weaken immune function (Hickey et al., 2003) and reduce growth rates. During this time, piglets are also more susceptible to illness due to a compromised immune system and insufficient nutrient intake (Kuller et al., 2004).

In our research the high occurrence of *S. aureus* (see Figure 3) was seen in 4 - 4.5 and 3 - 3.5 months old piglet groups, and in Farm C in these groups the occurrence of *S. aureus* is 28-37%, - higher than in pre-weaned group. However, other researchers (Smith et al., 2009) have found a trend that MRSA occurrence decreases when pigs from young piglets reach adult age.

In our research, we observed a tendency, that *S. aureus* distribution in pig farms in different age groups mostly differ because of animal welfare and hygiene conditions. In 3 - 3.5 months old piglet group, *S. aureus* occurrence is one of the highest (92% in Farm C and 63% in Farm A). During that period in all farms pigs were transported from the piglet barn to pig fattening barn. In Farm B in the age group of 3 - 3.5 months old piglets, *S. aureus* were not found. In Farm B that group was provided with strain and other environment enrichment things, consequently, stress signs were low and cannibalism signs were no evident. In Farm B pre-weaned piglets got antibiotics less than other groups, and therefore *S. aureus* occurrence was the highest there. Nevertheless, *S. aureus* occurs despite the usage of antibiotics in a non-stop regime in low dosages.

To the authors' knowledge, this is the first examination of *S. aureus* occurrence in sows' milk in Latvia. We found *S. aureus* in 13% samples of milk, usually in sows with signs of mastitis, and that is one of the ways how a sow can infect newborn piglets, therefore not always, when sows were infected with *S. aureus,* the microorganism appeared in milk. Comparing our data to other researchers' data about dairy cow herds (Sommerhauser et al., 2003), where occurrence of *S. aureus* in milk samples varied from 9-27% depending on herd, our data includes in this interval one of lowest rates and it shows, that *S. aureus* occurrence in sows' milk is similar to occurrence of S. *aureus* in cows' milk.

S. aureus was found only in 3 of 23 air samples in pig groups with the highest *S. aureus* occurrence,

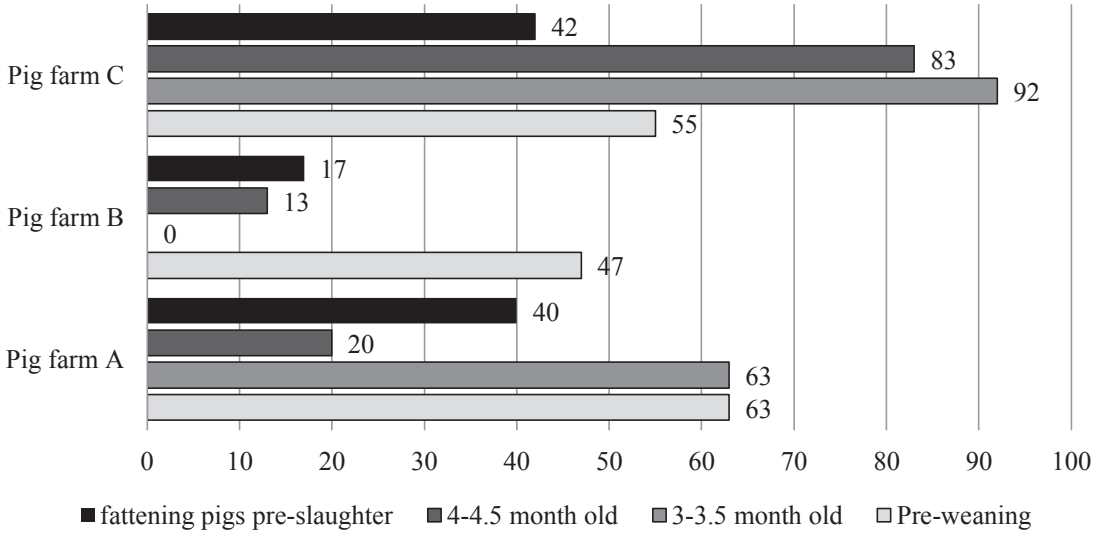

Figure 3. *S. aureus* distribution % in different pig age groups in pig farms.

but in other researchers' investigation MRSA was found in 21 samples of 24 (Schulz et al., 2011) and in 23 samples of 27 (Friese et al., 2011). In J. Schulz et al. research occurrence of MRSA in the investigated farms in pig nasal samples was 80%, but in our study *S. aureus* occurrence was only 34%. That shows a tendency: the higher the occurrence of microorganism in the herd population, the more spread it is in the environment, including air.

Further investigations are necessary to find MRSA in the isolated *S. aureus*.

Conclusions

1. Occurrence of *S. aureus* in the Latvian pig farms was 41% and the highest occurrence of *S. aureus* was among 3-3.5 months old piglets.
2. *S. aureus* was 1.8 times more frequently found in nasal than in rectal samples, and only in 28.4% of pigs *S. aureus* was found in both - nasal and rectal samples.
3. *S. aureus* was found in sows' milk and air in 13% of samples.

References

1. Beattie V., Walker N., Sneddon I. (1995) Effects of environmental enrichment on behaviour and productivity of growing pigs. *Journal of Animal Welfare,* Vol. 4, pp. 207 – 220.
2. Boer E., Zwartkruis-Nahuis Wit B., Huijsdens X., Neeling A., Bosch T., Oostero R., Vila A., Heuvelink A. (2009) Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International Journal of food microbiology,* Vol. 134, pp. 52 – 56.
3. Boucher H., Miller L., Razonable R. (2010) Serious Infections Caused by Methicillin-Resistant *Staphylococcus aureu*s. *Clinical Infectious Disease,* Vol. 51, pp. 183 – 197.
4. Brown D., Edwards D., Hawkey P., Morrison D., Ridgway G., Towner K., Wren M. (2005) Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureu*s (MRSA). *Journal of Antimicrobial Chemotherapy,* No 18, pp. 1000 – 1018.
5. Carr J. (1998) *Garth Pig Stockmanship Standards*, 5M Enterprises, London, UK, 64 p.
6. Charlier C., Crentenet M., Even S., Loir Y. (2009) Interactions between *Staphylococcus aureus* and lactic acid bacteria: An old story with new perspectives. *International Journal of Food Microbiolog,* Vol. 131, pp. 30 – 39.
7. Council Directive (EC) No 2008/120/ of 18 December 2008 Laying down minimum standards for the protection of pig (2009). *Official Journal of European Union,* No 52, pp. 5 – 13.
8. Dewaele I., Man I., Delputte P., Butaye P., Vlaemynck G., Herman L., Heyndrich M., Rasschaert G. (2013) Methicillin-resistant S*taphylococcus aureus* (MRSA) on Belgian pig farms. Available at: http://www2.llu.lv/research_conf/Formatting.pdf/, 2 March 2014.
9. Assesment of the public health significance of methicillin resistant *Staphylococcu aureu*s (MRSA) in animal and foods (2009) European Food Safety Aauthority. Available at: http://www.efsa.europa.eu/de/scdocs/doc/993.pdf/, 2 March 2014.
10. Ekkel E.D., Savenij B., Schouten W.G., Tielen M.J. (1996) Health, welfare, and productivity of pigs housed under Specific-Stress-Free conditions in comparison with two-site systems. *Journal of animal science.* No 74, pp. 2081 – 2087.
11. Friese A., Schulz J., Hoehle L., Hartung J., Rosler U. (2011) MRSA in air of german breeding and fattening pig farms. In: Animal Hygiene Sustainable Livestock Production, XV ISAH Congress 2011, International Society for Animal Hygiene, Vienna, Austria, pp. 515 – 517.
12. Hickey M., Drennan M., Earley B. (2003) The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute phase proteins and in vitro interferon gamma production. *Journal of Animal Science*. No 81, pp. 2847 – 2855.
13. Jonge De F., Bokkers E., Schouten W., Helmond F. (1996) Rearing pigs in a poor environment: developmental aspects of social stress in pigs. *Journal of Physiology Behaviour,* No 60, pp. 389 – 396.
14. Kock R., Harlizius J., Bressan N., Laerberg R., Wieler L., Witte W., Deurenberg R., Voss A., Becker K., Friedrich A. (2009) Prevalence and molecular characteristics of methicillin-resistan *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals. *European Journal of Cilinical Microbiology and Infectious Diseases,* No 11, pp. 1375 – 1382.
15. Kuller W., Soede N., van Beers H., Schreurs P., Langendijk M., Taverne J., Verheijden H., Kemp B. (2004) Intermittent suckling: Effects on piglet and sow performance before and after weaning. *Journal of Animal Science,* Vol. 82, pp. 405 – 413.
16. Morgan M. (2008) Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? *Journal of Antimicrobial Chemotherap,* No 62, pp. 1181 – 118.

17. Muirhead M.R., Aleksander J.L.T., Carr J. (2013) *Managing Pig Health: A Reference for the Farm.* 2nd edition, Carr J., London, UK, 600 p.
18. Smith C., Male J., Harper A., Kroeger J., Tinkler G., Moritz E., Capuano W., Herwald L., Diekama D. (2009) Methicillin resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in Mildwestern U.S. swine and swine workers Available at: http://www.plosone.org/article/info %3Adoi%2F10.1371%2Fjournal. pone.0004 25 8#pone-0004258- g0 02/, 2 March 2014.
19. Schulz J., Friese A., Rosler A., Hartung J. (2011) Detection of airborne MRSA in and around farms. In: Animal Hygiene Sustainable Livestock Production, XV ISAH Congress 2011, International Society for Animal Hygiene, Vienna, Austria, pp. 523 – 524.
20. Sommerhäuser J., Kloppertt B., Wolter W., Zschöck M., Sobiraj A., Failing K. (2003) The epidemiology of *Staphylococcus aureus* infections from subclinical mastitis in dairy cows during a control programme. *Veterinary Microbiology,* Vol. 96, pp. 91 – 102.
21. Weese J., Duijkeren E. (2009) Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudonermedius* in veterinary medicine. *Veterinary Microbiology,* Vol. 140, pp. 418 – 429.