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Microarray based genetic profiling of Staphylococcus aureus isolated from abattoir byproducts of pork origin

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Introduction

Roughly 23 million tons of pork meat are processed in the European Union annually with a rising tendency. A significant proportion of this meat is wasted during processing either due to shortcomings in the handling of sidestreams or due to low consumer acceptance and therefore limited marketability of products. In other parts of the world, especially various Asian regions, pig ear or pig tongue and other byproducts are considered a delicacy of great value. Also, in Europe, the movement of "nose to tail" eating has gained recognition in gastronomy and among the general public in recent years. It aims at utilizing all parts of an animal, giving special attention to the culinary potential of offal. Currently, information on the safety of such products is limited, and information on the occurrence of *Staphylococcus aureus* is missing. *S. aureus* is a common skin colonizing organism responsible for staphylococcal food poisoning (SFP). In 2015, EFSA reported 434 food-borne outbreaks due to staphylococcal enterotoxins (SE). Of these, 85 outbreaks were associated with meat or meat products. Generally, pork meat production has raised concern due to the transmission of livestock associated- methicillin-resistant *S. aureus* (LA-MRSA) from animals to humans. The most prevalent MRSA lineage in Europe is CC398, while in Asia CC9 is more frequent. The genetic profiles of *S. aureus* isolated from neck, belly, back, and ham of pig carcasses in Switzerland have been reported, but little is known about the occurrence of *S. aureus* on slaughtering byproducts.

In this study, ear, forefoot, heart, intestine, liver, rib bone, sternum, bladder, stomach, hind foot and tongue of porcine origin were screened for *S. aureus* and the detected isolates were further characterized. In order to unravel the genomic population structure of *S. aureus* isolates, *spa* typing and DNA microarray analysis were used.

The objectives of this study were to determine the prevalence of *S. aureus* found on abattoir byproducts of pork origin and to characterize their virulence gene and antibiotic susceptibility profiles.

Material and Methods

Overall, 524 items of abattoir byproducts of pork origin such as ear, forefoot, hind foot, heart, intestine, liver, rib bone, sternum, bladder, stomach and tongue from different abattoirs were screened for *S. aureus*. DNA microarray was performed using Staphytype genotyping kit 2.0 (Alere). In addition, the sequence of the polymorphic X region of the *spa* gene of each *S. aureus* isolate was determined (*spa* typing).

Results

Overall, 40 (0.08%) of the 524 sampled byproducts were positive for *S. aureus*. Parts with the highest prevalence were tongue (0.29%) and ear (0.24%), followed by rib bone (0.13%), sternum (0.09%), heart (0.07%) forefoot (0.02%) and liver (0.02%).

Of the 40 isolates obtained from pork byproducts, 39 could be assigned to a total of six clonal complexes (CC). The most prevalent CCs were CC9 (27.5%), CC1 (22.5%) and CC7 (22.5%). An attribution of CCs to the respective source of isolation (body part) showed no difference between CCs present at outer body parts and those on inner organs. It could be hypothesized that inner organs were contaminated during meat processing. This is supported by the fact that not all CCs were found in all abattoirs.

Twelve *spa* types were associated with the samples. The most frequent *spa* types were t091 (n = 9), t1491 (n = 8), t899 (n = 6) and t034 (n = 5).

Among the tested antibiotic resistance genes, *blaZ/I/R*, *qacC*, *fosB*, *vgaA*, *tetK/M*, and *aacA-aphD* were found. CC398 appeared to exhibit the most heterogeneous resistance profile compared to other complexes. For CC398 isolates, resistance genes *blaZ/I/R*, *vgaA* and *tetK* as well as *tetM* were detected. No MRSA strains were detected among the *S. aureus* strains investigated in this study.

The studied set of isolates displayed a variety of enterotoxin genes, which were heterogeneously distributed within clonal complexes and *spa* types. *Sea* (N315) was present in CC1, CC7, CC49, and CC398. The gene coding for enterotoxin B (*seb*) was found in CC1, CC9, CC30, and CC398. The *seh* gene was distributed across CC1, CC7, CC9, CC30, and CC398. The most prevalent toxin genes were the *egc* encoded genes *seg*, *sei*, *sem*, *sen*, *seo* and *seu*, which were detected in 11 strains belonging to CC1, CC7, CC9, CC49, and CC398. Interestingly, only one strain isolated from a heart harbored the *sec* and *sel* genes. Only two strains (*spa* types t015 & t7439) did not harbor any of the tested enterotoxin genes.

SplitsTree analysis revealed an association of certain CCs and abattoirs. No association of CCs with particular body parts or outer/inner organs was observed. However, *S. aureus* from certain body parts were associated with certain abattoirs, e. g. *S. aureus* were only detected in sternum and rib bone samples originating from one abattoir. It could be hypothesized that such isolates stem from post mortem contamination during the slaughtering and meat handling process, rather than from the animal source.

Discussion and Conclusion

Sampling of pork byproducts in Switzerland demonstrated low prevalence of *S. aureus*. Microarray based genetic profiling of 40 *S. aureus* strains revealed a diverse population structure. No MRSA were detected. A variety of enterotoxin genes was found distributed over almost all clonal complexes. Overall, the isolates did not differ significantly from those found in previous studies in pork meat. Therefore, the investigated pork byproducts do not pose a greater health threat to consumers than conventional pork products with regard to *S. aureus*. Our findings suggest that occurrence of *S. aureus* on byproducts was linked to contamination during the slaughtering process in some abattoirs. Adequate handling of these processing sidestreams should ensure proper quality and therefore minimize product loss.