7 Summary

Investigations on diagnosis and epidemiology of *Staphylococcus aureus* in dairy herds in Brandenburg

The objective of this study was to analyse the epidemiological features of *Staphylococcus (S.) aureus* by using genotyping and antibiogram typing. Furthermore different methods for identification of *S. aureus* were performed to compare the methods' ability to identify *S. aureus* from bovine milk.

Milk samples were collected from six dairy herds with high prevalence of *S. aureus* in the federal state of Brandenburg, Germany. Of each herd, 32 cows in different stages of lactation and different age groups were chosen for sampling.

Using Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) and disk diffusion assay between 20 and 22 *S. aureus* isolates were typed per herd.
RAPD-PCR was performed using two different primers (1. (5´-d[GTTTCGCTCC] -3´) and 2. (5´-d[AACGCGCAAC]-3´)). A panel of 12 antibiotics was used in the disk diffusion assay. The fingerprints of the *S. aureus* strains were compared within herds, not between herds to analyse the distribution of strains within herds.
Using RAPD-PCR the number of *S. aureus* strains found in each herd varied between two and six. In each herd one of the strains could be identified as numerically dominating. This strain accounted for 54,5% to 95,5% of the analysed isolates.
S. aureus strains obtained from milk samples of primiparous cows did not differ from *S. aureus* strains obtained from milk samples of multiparous cows. The dominant strain of a particular dairy herd was found in cows of different stages of lactation and different age groups.
The numerically marginal strains (only one or two isolates) were mainly found in milk samples of multiparous cows.
Of all *S. aureus* strains isolated from multiparous cows 18,7% (14 out of 75) and of all *S. aureus* strains isolated from primiparous cows 5,5% (3 out of 55) were found only once or twice in a particular dairy herd.

Using antibiogram typing in three of the six dairy herds all *S. aureus* isolates were categorized as identical in terms of resistance features. In two more dairy herds the *S. aureus* isolates were categorized in 2 and 3 groups with identical resistance features, respectively. Only one dairy herd showed five different groups in terms of resistance features. There was no conformity in the results of the applied typing methods RAPD-PCR and diffusion disc method.

The diagnostic methods applied were hemolysis, tube coagulase testing, anaerobic fermentation of mannitol, growth on agar supplemented with acriflavin, production of acetoin and two commercially available latex agglutination tests (Staphylase-Test ® (Oxoid) and Slidex Staph Plus ® (bioMerieux)).
The testing panel consisted of 269 *staphylococcus* isolates.
A particular isolate was categorized as *S. aureus,* provided that all of the following conditions were met: it showed a positive tube coagulase test, anaerobic fermentation of mannitol and growth on agar supplemented with acriflavin.
Applying these conditions 193 (71,7%) of all isolates were identified as *S. aureus,* whereas 72 (26,8%) were identified as coagulase negative staphylococci (CNS). Four isolates (1,5%) showed positive tube coagulase test but could not be identified as *S. aureus.*
The sole occurrence and the type of haemolysis was no sufficient criterion in this study to identify an isolate as *S. aureus*. Although 99,3% of all isolates with double hemolysis could be categorized as *S. aureus*, only 76,6% of all isolates categorized as *S. aureus* showed double hemolysis. Although 98,4% of all isolates categorized as *S. aureus* showed beta-hemolysis (beta- or double hemolysis), only 91,8% of all isolates showing beta-hemolysis could be categorized as *S. aureus.* 16 (22,2%) of the identified 72 CNS isolates showed also beta-hemolytic activity.
The main problem of the application of the two rapid identification kits proved to be the occurrence of non-classifiable results. The Staphylase-Test ® failed in categorizing 6,7% of all analysed staphylococci as either *S. aureus* or CNS, the Slidex Staph Plus ® failed in categorizing 7,1%.
The production of acetoin was applied to only 142 isolates, because its responses could be interpreted ambiguously and it became obvious, that the overall results would differ considerably from the results of the other testing methods.

According to the results of this study, staphylococci presenting double hemolysis can be classified with a high accuracy as *S. aureus*. Staphylococci showing no hemolytic activity can be classified with a high accuracy as non-*S. aureus* staphylococci. Isolates showing beta-hemolysis need to be further examined by coagulase test.