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Molecular epidemiology of methicillin-resistant *S. aureus* (MRSA) in veterinary medicine

(Molekulare Epidemiologie Methicillin- resistenter *S. aureus* (MRSA) in der Veterinärmedizin)

Various populations of *S. aureus*, which have established themselves in the hospital environment over the last decades, have acquired different resistance factors and are now difficult to combat, even in the field of veterinary medicine. In particular, Methicillin-resistant *Staphylococcus aureus* (MRSA) are one of the most predominant causes of sporadic and endemic infections in hospitals as well as in hospital-adapted institutions.
Since the early `70s, infections due to MSRA have also been reported in animals. In the last years, however, there have been increasing reports about MRSA infections in small animals and horses. Recently published studies address the potential of interspecies transferability, or the zoonotic character of MRSA.
The aim of this work was to compile data and knowledge about the occurrence and relevance of MRSA in the field of veterinary medicine. Therefore, four main goals were defined prior to this work. First of all, a comparative investigation of different methods for phenotypical identification of MRSA of animal origin was performed. A second point of interest was the recording of data concerning occurrence, patterns of resistance and characterization of relevant genetic markers. In close cooperation with the clinic and policlinic for small animals of the Free University of Berlin, a third goal was set: To pinpoint the zoonotic potential of some MRSA lineages and detection of potential nosocomial transmission pathways in a clinical setting. The last intention of this investigation was to analyze all recorded data from an epidemiological point of view. Therefore, isolates originating from animals were compared to a set of data from human MRSA strains and assigned to a database that provided information about several *S. aureus*-genotypes.
International recommended methods were applied to characterize the MRSA isolates in this work. Clonal analysis was performed by employing endonuclease *Sma*I digestion, followed by pulsed-field gel-electrophoresis (PFGE) in order to compare the genomic marcrorestriction patterns of all isolates. Multilocus sequence typing (MLST), which is based on the sequence analysis of defined sections of seven housekeeping genes, enabled the assignment of sequence types (ST) to each MRSA and into the whole *S. aureus* population analysed so far (www.mlst.net). Typing of the mobile genetic element (SCC*mec*), which harbours the *mec*-complex in staphylococci, was carried out using a previously published Multiplex polymerase chain reaction (PCR) strategy. Unfortunately, this PCR typing did not always produce satisfying results.
A total of 1,544 specimens (including 144 isolates from other veterinary facilities) from animals, humans and everyday objects were examined in this work.
Comparing different phenotypical methods to diagnose MRSA from animal origin revealed that the agar diffusion test with Cefoxitin (30ug) seems to be superior to other methods, e.g. agar diffusion test with Oxacillin 1µg and 5µg as well as an Oxacillin screening agar. Despite these results, the best method for MRSA verification still is PCR, detecting the *mec*A gene that codes for the resistance determinant as well as a species-specific marker like the *nuc* gene and/or a specific 16S rDNA sequence.
Samples from 135 *S. aureus* infected horses were sent in from different veterinary microbiological institutions in Germany. Upon examination, 70 samples proved to contain *S. aureus*, 11 of those turned out to be positive for MRSA (15.7%). By applying PFGE, three clonal types were identified and designated as "A", "B", "C". Surprisingly, these clonaltypes

showed a degree of genetic similarity of 86%. A second examination emphasized these findings, proving this close relationship. The detected STs, determined by MLST, turned out to be the closely-related ST8 and ST254, which differ from each other only by substitution of a single base-pair (bp) in one of the seven housekeeping genes sequenced (*arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi, ypiL*).
Unfortunately, the restricted number of horse isolates investigated here is a limiting factor in the interpretation of these results. All recorded staphylococcal chromosomal cassettes (SCC*mec*) from equine MRSA isolates in this work proved to be SCC*mec* IV.
Additional 6 MRSA isolates of small animals from different sources in Germany showed different PFGE types: "F", "D" and "H" as well as subtypes. The obtained sequence types determined by MLST were ST254, ST225 and ST239. While five isolates proved to contain SCC*mec* IV, one isolate (out of those related to ST225) was positive for SCC*mec* II.
In the third part of this work, during an investigation period of 20 months, *S. aureus* was found in 6.9% (60/866) of all clinical samples being sent to our laboratory for diagnostic purposes from the small animal hospital. Twenty-six of these tested positive for *mec*A (including isolates from exotic animals such as parrots, turtles as well as a bat). In addition, we collected and investigated nasal specimens from dogs (257 specimens of 191 animals) and humans (tested three times: n=62; n=62; and n=88). Furthermore, 20 samples of everyday objects were analysed. MRSA was detected in six canine cases, in nasal specimens from 20 persons, and on 3 objects. Two different PFGE types dominated in this setting during the investigation period, namely type "G" and "D". MLST analysis showed two genetically different MRSA clones: PFGE type "G" corresponded to ST22, and PFGE type "D" was determined to ST239. Despite the SCC*mec* IV, which was found to be related to type "D", the utilized SCC-typing techniques used in this work failed in typing SCC*mec* in PFGE type "G".
In regard to epidemiological aspects, evaluating data from this small animal hospital indicated that an occasional nosocomial spread of the aforementioned MRSA clones may have occurred within this institution.
The final part of this investigation was to classify all MRSA ST which had been identified during this work, in an epidemical context by using additional data from www.mlst.net in performing a Minimum spanning tree analysis. This approach could demonstrate that all MRSA STs in this thesis (ST8, ST239, ST254, ST225, ST22) have also been frequently observed in isolates from human MRSA infections with an almost global occurrence.
The data presented here are evidence of nosocomial infections due to MSRA in veterinary medicine facilities. Due to the constant adaptation processes of infectious agents to their environment and their high adaptability to host organisms of different (animal) species, the development of standardized hygienic guidelines for veterinary practice and hospitals should be enforced. Especially the potential nosocomial transmission of MRSA from animal to animal as well as between human and animal should be the focus of hygienic measures as well as future research.
The current relevance of MRSA infections in animals as a potential source and/or reservoir for MRSA mediated infections in humans is still unknown. The apparent ready transferability of MRSA, in particular in an environment with a certain selection pressure (practice, hospital), showed that the risk for nosocomial transmission between human and animal with close contact and/or lack of hygienic measures must be taken seriously.