Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an emerging public health problem worldwide, no longer only associated with healthcare-associated infections. With the exception of some recent reports concerning infections in cats, dogs and horses, infections with MRSA in companion animals have been infrequently reported. Here we submit findings for MRSA infections in horses in a central European university veterinary hospital.

**Introduction**

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a worldwide public health problem [1,2]. Increasing prevalence of healthcare-associated MRSA infections is usually associated with a wide dissemination of particular epidemic clonal lineages of the *S. aureus* population [3]. Since the late 1990s, MRSA has emerged in many countries as a cause of invasive skin infections in the community, independently from the healthcare setting [4-8]. In this context, colonisation and infections with MRSA in domestic animals are of particular interest with regard to a mutual dissemination between humans and animals. The first communication on MRSA infections in domestic animals concerned mastitis cases in dairy cows in Belgium in 1972 [9]. Since that time there have been reports of sporadic cases of infection with MRSA in a variety of other domestic animal species such as horses, chickens, dogs and cats [10-13]. MRSA infections in horses associated with wide dissemination of a particular clonal lineage have been recently documented in Canada [14,15].

Here we report on emergence of MRSA in a university veterinary hospital and on an assessment of the relation of human and animal MRSA isolates by means of molecular typing. This includes Smal macrorestriction patterns, multilocus sequence typing (MLST) for assessing the core genome of *S. aureus* and characterisation of SCCmec elements of which at least 5 different groups have so far been described [16]. SCCmec (staphylococcal cassette chromosome mec) elements contain the meCA gene that codes for methicillin resistance [17].

**Materials and methods**

**Description of the setting:**

The Veterinary University of Vienna [Veterinärmedizinische Universität Wien (VUW)] consists of a large hospital with separate departments for small animals, horses, farm animals, reproduction and diagnostic imaging/laboratory diagnostics. On average, 23 000-24 000 domestic animals including horses, ruminants, pigs, dogs, cats and rodents are admitted to hospital for a variety of diseases each year. Within the equine department there are separate clinic buildings for orthopaedics, soft tissue surgery and internal medicine. When necessary for diagnostics and/or specialised treatment, animals are moved between different clinics. Furthermore, veterinarians undertaking postgraduate education are on duty in different departments, and move freely between the various clinic buildings.

**Origin of MRSA from infections and nasal colonisation in horses**

Clinical isolates (from 24 cases) were obtained from specimens for bacteriological diagnostics that were routinely submitted in cases of wound infections, infected joints and suspected infections of various organ systems from summer 2003 until spring 2005.

In order to investigate nasal colonisation, the both nostrils of 24 horses (4 with an MRSA infection, 20 without) that were treated by the orthopaedics department during the same time period in 2004 and 2005 were screened for MRSA by taking nasal swabs. Colonisation was found in only 1 of these animals.

**MRSA from nasal colonisation of VUW staff and veterinarians:** Specimens originated from direct cultures of swabs taken from both nostril.

**Reference strains for healthcare-associated epidemic MRSA**

These strains represent multilocus sequence types (ST) of the major clonal lineages of epidemic MRSA from Europe (ST22: 1678/96; ST05: 3391/02; ST247: 134/93; ST45: 1150/93; ST254: 993/93 and 1000/93). The strains were initially isolated from outbreaks of healthcare-associated infections and were established by representative Smal macrorestriction patterns and multilocus sequence types (MLST). These strains were included in the HARMONY collection of epidemic MRSA from Europe [18] and in the first MLST-based population study of MRSA from sources worldwide [3].

In the study described here, these reference strains were used for comparison of Smal macrorestriction patterns.

**Reference strains for community-acquired MRSA (CA-MRSA)**

ST80: 3925/02; ST01: 2773/03; ST30: 1880/04.

These strains represent multilocus sequence types of community-acquired MRSA that are frequently isolated in central Europe [6-8] and have been used in this study for comparison of Smal macrorestriction patterns. They originate from deep-seated skin infections in the community without hospital association, and are positive for the Panton-Valentineleukocidin determinants (*lukS*-*lukF)*.

**Methodology of specimen processing**

MRSA from infections in horses were obtained from direct cultures of swabs onto blood agar-plates. Colonies typical for *S. aureus* were subjected to species identification according to standard procedures [19] and were also evaluated for antimicrobial susceptibility [20]. Nasal colonisation swabs from the anterior of horses and of veterinary personnel were streaked onto blood agar plates and in parallel onto CHROM agar for MRSA from Becton-Dickinson. After incubation for 48 hours, at least five colonies that were suspected to be *S. aureus* were further subjected to species identification and antimicrobial susceptibility testing.
**Susceptibility testing**
First line testing in veterinary clinical microbiology was performed by disk diffusion assay [20]. All isolates exhibiting oxacillin resistance were subjected to microbroth assay for MIC determination [20] and to polymerase chain reaction (PCR) for the mecA gene.

**Molecular typing**
Smal macrorestriction patterns were obtained by use of the standardised HARMONY protocol [18] with subsequent cluster analysis based on the soft ware described by Claus et al [21]. For comparison of Smal patterns, cluster analysis was performed by comparing gel images.

For multilocus sequence typing (MLST) primers used and conditions of the PCR reaction corresponded to those described by Enright et al [3]. Sequences were analysed by use of the MLST databank (http://www.mlst.net).

**Characterisation of SCCmec elements by PCR**
PCR for cer-complexes, detection of type II and type III specific sequences and discrimination of type IV was performed as described by Witte et al [6].

**Demonstration of antibiotic resistance and virulence associated genes by PCR**
PCR for lukS-lukF was performed as described by Witte et al 2005 [5]. For PCR detection of genes conferring resistance to methicillin (mecA), oxacytetracycline (tetK, tetM), macrolides (ermA, ermB, ermC) and gentamicin (aac6’-aph2’), primers used and conditions were as in previous studies (Braulke et al [22] and Werner et al [23]). For PCR for superantigen determinants (tsf, eta, etb, etc) primers and conditions were used as described by Mehrotra et al 2000 [24].

**Results**

**Emergence of MRSA infections in horses:**
In 2003 there were 344 equine cases from which clinical specimens were submitted for bacteriological, diagnostics. *S. aureus* was isolated in 47 (14%) of these cases including 19 infections with MRSA. In 2004 samples from each of 29 among 259 cases were positive for *S.aureus* (11%) with 3 of them confirmed as MRSA infection. From January 2005 until April 2005 there were 21 *S.aureus* infections among 165 equine cases (13%), 2 of them were MRSA infections.

The time course, type of infection with MRSA and clinical department affected are shown in Figure 1. The index case occurred in surgery in mid 2003. Investigation into the introduction of MRSA from the community into the hospital via this patient was unsuccessful.

Currently, we have no information regarding cases of MRSA infections from other veterinary institutions in Austria. In this country the frequency of MRSA among *S. aureus* from healthcare-associated infections in humans is approximately 10%. This represents a relatively low incidence of infections when compared to the situation in other European countries [1]. Overall, the incidence of infections at the VUW with MRSA appears low considering the number of about 5000 horses admitted in 2004 and 2005, that means about 4.8 cases with an MRSA infection MRSA per 1000 admissions.

**Typing and comparative characterisation to MRSA from humans:**
All 24 isolates from infections horses exhibited similar Smal macrorestriction patterns with only minor variations that are still in the range of variability during the course of an epidemic (25). This pattern is consistent with intrahospital spread of one particular MRSA clone. These fragment patterns were different from those exhibited by healthcare-associated epidemic MRSA disseminated in Europe and from those of community-acquired MRSA [FIGURE 2].

Furthermore, there was no congruence when Smal-patterns of MRSA from horses were compared to patterns of 3680 MRSA isolates from healthcare-associated and community-acquired infections that were sent for typing to the author’s laboratory as the German National Reference Center for Staphylococci at the Robert Koch Institute between 2001 and 2004.

Five horse MRSA isolates that were subjected to MLST were identified as ST254.

PCR for typing of SCCmec elements that was performed on 5 isolates from horses revealed type IVd whereas IVc was found for MRSA of ST254 from humans [TABLE 1]. None of the investigated horse MRSA contained lukS-lukF, tst1, eta, etb or etc.

**Figure 1**
Emergence of 24 infections with MRSA in horses in different clinical departments from 2003 to 2005

**Figure 2**

Smal macrorestriction patterns of MRSA from infections in horses, shown together with Smal macrorestriction patterns of epidemic MRSA from healthcare-associated infections and of community-associated MRSA from central Europe

Reference strains for epidemic nosocomial MRSA and for community MRSA are indicated by code numbers and MLST types (STs in brackets)
Reference isolates as molecular mass standard S. aureus 8325: lane 0
Community-associated MRSA: lane 1: 3925/02 (ST80); lane 2: 2773/03 (ST01); lane 3: 1880/04 (ST30)
Epidemic healthcare-associated MRSA: lane 4: 1678/96 (ST22); lane 5: 3391/02 (ST05); lane 6: 134/93 (ST247); lane 7: 1150/93 (ST45); lane 8: 1000/93 (ST254); lane 9: 994/93 (ST254)
Horse isolates: lane 10: 1831/03 (ST254); lane 11: 762/04 (ST254); lane 12: 1457/03 (ST254); lane 13: 2576/03 (ST254)

<table>
<thead>
<tr>
<th>Year</th>
<th>Wound Infection</th>
<th>Fistula</th>
<th>Infected catheter</th>
<th>Infected joint</th>
<th>Tracheal lavage</th>
</tr>
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<tr>
<td>2003</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
<td>2005</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Clinical departments: S = surgery; O = orthopaedics; I = internal medicine
Transmission to human nasal colonisation of personnel and veterinarians:

During the time periods of emergence of MRSA infections in horses in the surgery and orthopaedic clinics in 2004 and 2005, nasal swabs from 43 people that were directly involved in treatment of horses and contained SCCmec IV elements. The MRSA isolates were identified as the same ST254 clone exhibiting the so called CA-MRSA-05 typing pattern that has previously been identified in healthcare-associated settings from humans and in part from horses [11].

Nasal colonisation of horses:

Data from human medicine indicates that nasal colonisation is an important reservoir with regard to infections of the primary carrier and to further dissemination [26,27]. A temporary colonisation (negative in a second investigation) was detected in only one among 24 horses. The MRSA isolate exhibited the same ST254 macrorestriction pattern as the isolates from infections and also contained a SCCmec IVd element.

Discussion

MRSA from infections in horses in a central European veterinary hospital exhibit MLST ST254. This type also exhibits the same ST254 macrorestriction patterns as isolates from infections in horses and contained SCCmec IVD elements.

Table

<table>
<thead>
<tr>
<th>Origin</th>
<th>MLST</th>
<th>No. of isolates investigated</th>
<th>Resistance phenotypes</th>
<th>Resistance genes</th>
<th>PCR characterisation of SCCmec-elements</th>
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</thead>
<tbody>
<tr>
<td>Horses, VUV</td>
<td>254</td>
<td>5</td>
<td>PEN, OXA, TET, GEN, TMP</td>
<td>mecA, tetM, aac6'-aph2′-IIV</td>
<td>IVd</td>
</tr>
<tr>
<td>Humans</td>
<td>254</td>
<td>5</td>
<td>PEN, OXA, ERY, CLI, TMP</td>
<td>mecA, ermA</td>
<td>IVc</td>
</tr>
<tr>
<td>Horses, Canada</td>
<td>8</td>
<td>1</td>
<td>PEN, OXA, ERY, CLI, GEN, OTE</td>
<td>mecA, ermC, aac6'-aph2′-IIV, tetM</td>
<td>IV</td>
</tr>
</tbody>
</table>

Transmission of MRSA of MLST ST254 from infections in horses in VUV compared with healthcare-associated MRSA of MLST ST254 from humans and to MRSA from infections in horses, Canada

MRSA isolates from horses were revealed as long term carriers (massive colonisation demonstrated in both a first investigation and follow-up sample 3 weeks later). The MRSA isolates exhibited the same ST254 macrorestriction patterns as isolates from infections in horses and contained SCCmec IVD elements.

Conclusion

Infections in horses with MRSA of MLST ST254 emerged independently of MRSA infections in humans. Although MRSA in horses may presently not represent a substantial reservoir for infections in humans in central Europe, further surveillance is needed with respect to human transmission and to emergence of new clonal lineages.

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References


Introduction

The epidemiology of invasive bloodstream pathogens has changed dramatically over the years [1-3]. The change in the incidence and epidemiology of infecting organisms has also brought about an increase in resistance to many antibiotic compounds [2,4,5]. Despite numerous publications on antimicrobial resistance, the comparison and evaluation of data is difficult, as the patient groups, sampling sites and infections involved in each study were different.

In order to overcome these problems, the European Antimicrobial Resistance Surveillance System (EARRS) began the collection of standardised data about the resistance of invasive isolates, focusing especially on Gram positive pathogens. Until 2005, information about Gram negative bacteria was available only in case of E. coli [6]. In addition, from the summer of 2005 onwards, data are being collected on Pseudomonas aeruginosa and Klebsiella pneumoniae [6]. Infections with Gram negative bacteria still constitute a topical problem in patients with invasive infections, which are quite frequent in Europe [7-13].