EMERGENCE OF MRSA INFECTIONS IN HORSES IN A VETERINARY HOSPITAL: STRAIN CHARACTERISATION AND COMPARISON WITH MRSA FROM HUMANS

C Cuny[1], J Kuemmerle[1], C Stanek[1], B Willey[2], B Strommenger[3], W Witte[3]

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.

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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 *Sma*I macrorestriction patterns, multilocus sequence typing (MLST) for assessing the core genome of *S. aureus* and characterisation of SCC*mec* elements of which at least 5 different groups have so far been described [16]. SCC*mec* (staphylococcal cassette chromosome *mec*) elements contain the *mec*A 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 *Sma*I 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 *Sma*I 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 *Sma*I macrorestriction patterns. They originate from deep-seated skin infections in the community without hospital association, and are positive for the Panton-Valentineleukocidin determinants (*luk*S-*luk*F).

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.

1. Veterinary University Vienna, Department V, Clinic of Orthopaedics, Austria
2. Department of Microbiology, Mount Sinai Hospital, Toronto, Ontario, Canada
3. Robert Koch-Institut, Wernigerode Branch, Wernigerode, Germany

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 *mec*A gene.

Molecular typing

*Sma*I 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 *Sma*I 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 *ccr*-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 *luk*S-*luk*F was performed as described by Witte et al 2005 [5]. For PCR detection of genes conferring resistance to methicillin (*mec*A), oxytetracycline (*tet*K, *tet*M), macrolides (*erm*A, *erm*B, *erm*C) and gentamicin (*aac*6'-*aph*2"), primers used and conditions were as in previous studies (Braulke et al [22] and Werner et al [23]). For PCR for superantigen determinants (*tst*, *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 *Sma*I 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].

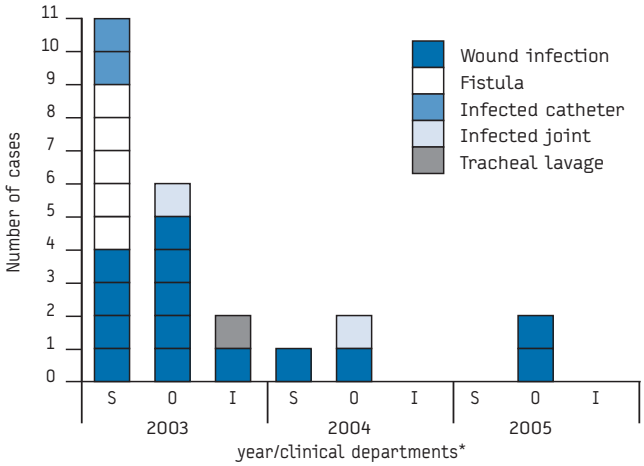
Furthermore, there was no congruence when *Sma*I-patterns of MRSA from horses were compared to patterns of 3680 MRSA isolates from healthcare-associated and community-acquired infections that were sent for typing to the author's laboratory as the German National Reference Center for Staphylococci at 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 SCC*mec* elements that was performed on 5 isolates from horses revealed type IVd whereas IVc was found for MRSA of ST254 from humans [TABLE]. None of the investigated horse MRSA contained lukS-lukF, tst1, eta, etb or etc.

FIGURE 1

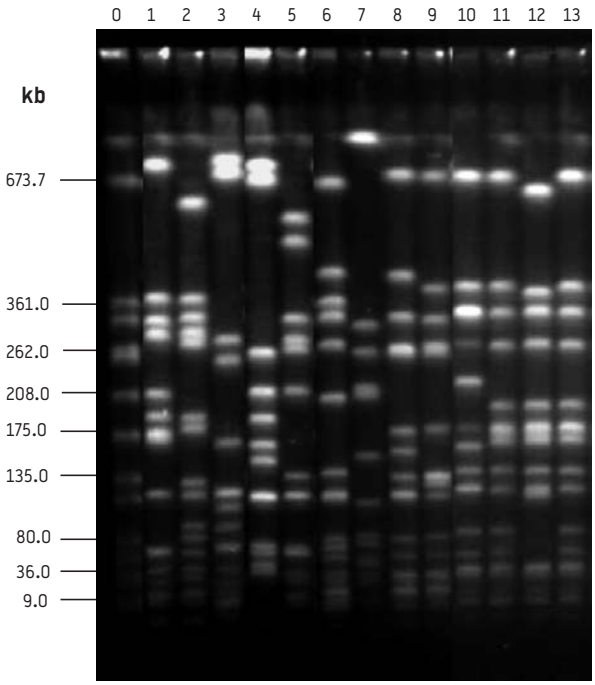
Emergence of 24 infections with MRSA in horses in different clinical departments from 2003 to 2005



* Clinical departments: S = surgery; O = orthopaedics; I = internal medecine

FIGURE 2

S*ma*I macrorestriction patterns of MRSA from infections in horses, shown together with S*ma*I 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

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

Origin	MLST	No. of isolates investigated	Resistance phenotypes	Resistance genes	PCR characterisation of SCC*mec*-elements
Horses, VUW	254	5	PEN, OXA, TET, GEN, TMP	mecA, tetM, aac6'-aph2"	IVd
Humans	254	5	PEN, OXA, ERY, CLI, TMP	mecA, ermA	IVc
Horses, Canada	8	1	PEN, OXA, ERY, CLI, GEN, OTE	mecA, ermC, aac6'-aph2", tetM	IV

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 animals (veterinarians, veterinary assistants, animal keepers) were investigated. Two veterinarians 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 *Sma*I macrorestriction patterns as isolates from infections in horses and contained SCC*mec* IVd elements.

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 *Sma*I macrorestriction pattern as the isolates from infections and also contained a SCC*mec* IVd element.

Discussion

MRSA from infections in horses in a central European veterinary hospital exhibit MLST ST254. This type has also been identified in healthcare-associated epidemic MRSA. This strain was frequent in the 1990s but has subsequently decreased in prevalence [28]. Reference isolate 994/93 is a representative of ST254 that was disseminated in the hospitals of the Order of Holy Elisabeth in the south-west of Germany and west of Austria [28]. A direct relationship between human MRSA of ST254 to those from horses is however unlikely,as both exhibit different *Sma*I-macrorestriction patterns and contain different subtypes of SCC*mec* IV elements. Subtypes of SCC*mec* elements of type IV differ by various DNA sequences in the region downstream from *mec*A. At the present time, no acquisition or loss of these sequences has been observed during the time course of dissemination of epidemic MRSA. PCR typing based on subtype specific DNA sequences appears to be a reliable tool for discrimination of subtypes. Demonstration of different subtypes of SCC*mec* elements in the genomic background of ST254 does however, not exclude an exchange of MRSA between humans and horses in the past. Another possibility is that methicillin-susceptible *S. aureus* of ST254 that was already widely disseminated among humans [29] was transferred to horses and later acquired a SCC*mec* element that is different from those acquired by human MRSA of ST254.

Until now MRSA exhibiting typing patterns like those of ST254 from horses have not been detected among MRSA isolates from infections in humans. Furthermore, the human ST254 strain has so far only been associated with healthcare-associated infections and has not emerged in the community.

However, the finding of stable nasal colonisation of two veterinarians who had been in contact with animals affected by MRSA infections demands further investigation of potential animal to human transmission.

This is underlined by findings of MRSA among horses that were reported from Canada. In this report a single well-recognised MRSA clone exhibiting the so called CA-MRSA-05 typing pattern that previously had been identified in healthcare-associated settings from 5 different geographical sites in Canada was demonstrated to have the ability to colonise the nose of horses. This clone spread among both horses and humans on farms and among personnel in veterinary hospitals (14,15). When representative isolates of these MRSA strains were subjected to MLST, we found it to be ST 8 [TABLE]. MRSA strains exhibiting ST8 are widely disseminated in US hospitals and may also become more frequent in Canada since a population dynamics of *S. aureus* and in particular of MRSA is well known [30]. Furthermore, MRSA of this clonal lineage containing the *luk*S-*luk*F gene that confers enhanced virulence became prevalent as community-acquired MRSA in the US [31], and sporadic cases of infections in the community have also been reported from Norway [32].

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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ORIGINAL ARTICLES

Surveillance report

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE OF INVASIVE PATHOGENS: THE ESTONIAN EXPERIENCES

K Lõivukene[1], K Kermes[1], E Sepp[2], V Adamson[3], P Mitt[3], Ü Kallandi[4], K Otter[4], P Naaber[1], on behalf of the European Antimicrobial Resistance Surveillance System, Estonian Study Group*

The aim of the present study was to evaluate the needs for surveillance of invasive Gram-negative pathogens in Estonia. The antimicrobial susceptibility data of invasive isolates of *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella spp, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *enterococci* were collected in accordance with EARSS (European Antimicrobial Resistance Surveillance System) protocols. Despite the higher rate of Gram positive pathogens, their resistance was low in contrast to the elevated resistance established for Gram negative pathogens. The higher resistance to antimicrobials was particularly associated with *A. baumannii* and *P. aeruginosa*. Also, the proportion of extended-spectrum beta-lactamases (ESBL)-producing strains was 23% among *Klebsiella spp.* and 3.6% among *E. coli*. The inclusion of invasive Gram negative pathogens in antimicrobial resistance surveillance provides useful information concerning local pathogen susceptibility, as well as for the empirical treatment of suspected infections.

1. Laboratory of Clinical Microbiology, United Laboratories of Tartu University Clinics, Tartu, Estonia
2. Department of Microbiology, University of Tartu, Estonia
3. Infection Control Service, Tartu University Clinics, Tartu, Estonia,
4. AstraZeneca, Estonia

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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 (EARSS) 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].