Evolution of Multidrug-Resistant *Staphylococcus aureus* Infections in Horses and Colonized Personnel in an Equine Clinic Between 2005 and 2010

Sandra Sieber, Vinzenz Gerber, Vendula Jandova, Alexandra Rossano, John Marc Evison, and Vincent Perreten

A total of 70 *Staphylococcus aureus* isolates from postoperative infections in hospitalized horses were isolated between January 2005 and January 2011. Among them, 12 isolates were methicillin-susceptible *S. aureus* (MSSA), 18 were borderline-oxacillin-resistant *S. aureus* (BORSA), and 40 were methicillin-resistant *S. aureus* (MRSA). During the same period, the equine clinic personnel were screened for nasal carriage of BORSA and MRSA. Genotyping revealed that BORSA ST1 (MLST)-t2863 (spa) isolates were responsible for most equine infections and were the main isolates found in colonized members of the personnel between 2005 and 2007, and that in 2007, MRSA ST398-t011-IVa SCC mec emerged in infection sites and personnel, replacing BORSA. Besides decreased susceptibility to oxacillin, all MRSA and BORSA of these two major clonal lineages displayed resistance to gentamicin and kanamycin conferred by the aac(6′)-Ie-aph(2′)-Ia gene and to trimethoprim conferred by dfr(K) in MRSA and dfr(A) in BORSA. All MRSA had additional resistance to tetracycline conferred by *tet(M)*, whereas BORSA generally also display resistance to streptomycin conferred by *str*. The number of hospital-acquired MRSA infections in horses could be limited after the introduction of basic hygiene measures and personnel decolonization. Two MRSA carriers could not be decolonized using mupirocin, and a year after decolonization, additional members were recolonized with MRSA. Hygiene measures should, therefore, be maintained to limit the transmission of *S. aureus* between personnel and horses.

**Introduction**

Bacterial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have increasingly become problematic in equine clinics. Specific MRSA belonging to clonal complexes CC1, CC5, CC8, CC22, CC59, CC88, CC398, and mainly to spa type t002, t008, t009, t011, t020, t022, t032, t034, t036, t064, t127, t166, t186, t216, t451, t588, t1197, t1451, and t2123 have been identified as a cause of nosocomial infections in numerous equine clinics. S. aureus can colonize the skin and mucosa, especially nasal mucosa, of healthy horses and humans, which then acts as a reservoir for MRSA and contributes to its spread into the community and hospitals. Horses entering the hospital have been shown to contribute to the introduction and spread of MRSA in the clinic. Once present in the hospital environment, MRSA may persist and become responsible for increasing rates of nosocomial infections. Colonized veterinary personnel can potentially transmit MRSA to humans or to their equine patients and can contaminate materials. MRSA was detected in rooms where veterinary students and staff member gather. Hand hygiene is considered to be one of the most important infection-control tools in human medicine and has also been shown to limit the number of infections with MRSA in equine clinics.

Bacterial infections of horses have been routinely screened at the Equine Clinic of Bern, Switzerland. From the year 2005, there was an increasing number of *S. aureus* infections with borderline-oxacillin-resistant *S. aureus* (BORSA) and MRSA. BORSA are characterized by decreased susceptibility to oxacillin at a minimum inhibitory concentration (MIC) of 1–2 mg/L, which may result from either overexpression of the β-lactamase gene *blaZ* or mutations in penicillin-binding-proteins PBP1, 2, or 4. Unlike MRSA, BORSA does not contain an alternative penicillin-binding protein PBP2a. The PBP2a of MRSA is specified by the gene.
meCA, which is situated on a transferable Staphylococcus Cassette Chromosome meC (SCCMec).\textsuperscript{10,15} BORSA and MRSA generally also contain additional antibiotic resistance genes and display a multidrug-resistance profile.\textsuperscript{3,10,31} This study describes the evolution of infections with BORSA and MRSA in horses and nasal carriage among personnel in the Equine Clinic of Bern during a 6-year period. The genetic characterization of these multidrug-resistant \textit{S. aureus} isolates should determine whether specific clones were responsible for nosocomial infections and whether clinic personnel contributed to the introduction and the spread of these pathogens into the clinic. The results here also served as a baseline for evaluating newly introduced adequate hygiene measures and evaluating the effects of personnel decolonization of MRSA on the control of infections in the Equine Clinic of Bern.

**Materials and Methods**

**Sample collection**

Between January 1, 2005, and January 31, 2011, all horses (\(n = 686\)) suffering from a postoperative purulent infection or thrombophlebitis were sampled with swabs (Oxoid Ltd., Basingstoke, Hampshire, England) at the University Equine Clinic of Bern. Clinical information was obtained from the Equine Clinic database using OblonData software (Amacker & Partner Informatik, Zurich, Switzerland). All clinical samples from horses were taken from ichor. Only the first sample per patient was considered in the data analysis, and horses with recurrent infections were not included. The number of horses that underwent surgery, the number of postsurgery infections, and the number of samples taken from infected sites during this period are listed in Table 1. The prevalence of \textit{S. aureus} in infected sites was calculated by dividing the number of infection sites containing \textit{S. aureus} by the number of samples taken from ichor. The nasal cavities of 135 horses entering the Equine Clinic over a 3-month period (January to March 2008) were also tested for BORSA and MRSA using swabs to estimate carriage on admission.

All the equine clinic personnel, consisting of veterinarians, anesthesiologists, technicians, and students on clinical rotations, participated spontaneously in the study and were tested five times between 2006 and 2010 for the presence of BORSA and MRSA using nasal swabs premoistened in physiological saline solution (0.85% NaCl) (Table 1). The variable number of sampled people was due to the rotation of students and residents. In 2008, staff from the radiology wards were also included. The prevalence of MRSA and BORSA carriage in the personnel was calculated by dividing the number of carriers by the number of persons sampled. During the follow-up after the decolonization treatment of the personnel, samples were taken from the nose, throat, and the inguinal region as previously described.\textsuperscript{26}

**Isolation and identification of staphylococci**

Bacteria were cultivated from swabs on trypton-soya-agar plates containing 5% sheep blood (Becton, Dickinson & Company, Franklin Lakes, NJ) for 24–48 hr at 37°C. \textit{S. aureus} isolates were identified by hemolysis, Gram-staining, catalase activity, and using SaSelect chromogenic agar (Bio-Rad Laboratories AG, Reinach, Switzerland). \textit{S. aureus} colonies
were spread onto MRSA selective agar (MRSA II; Becton, Dickinson & Company). On this agar, BORSA growth was not inhibited, and small colonies appeared after 24 hr of incubation at 37 C. MRSA were confirmed by the detection of the mecA gene by PCR.\textsuperscript{34}

**Antimicrobial susceptibility testing**

MICs of antibiotics were determined by broth microdilution in Mueller-Hinton using custom Sensititre susceptibility plates, model NLV73 (Trek Diagnostics Systems, East Grinstead, England; MCS Diagnostics BV, Swalmen, The Netherlands), and according to CLSI guidelines.\textsuperscript{5} Antibiotic resistance genes were detected using a microarray.\textsuperscript{33}

**Genotyping**

Genomic DNA was isolated using the peqGOLD Bacterial DNA Isolation Kit (Classic Line, PEQLAB Biotechnologie GmbH, Erlangen, Germany). Genetic relatedness was determined by multilocus sequence typing (MLST), spa typing, SCC\textit{mec} typing, and variable-number-tandem-repeat (VNTR)-based method. Sequence types (ST) were determined as previously described\textsuperscript{11} and were assigned using the MLST-Homepage (http://saureus.mlst.net). Spa type was determined as previously described and analyzed using the Ridom StaphType software (Ridom StaphType, Ridom GmbH, Würzburg, Germany).\textsuperscript{30} SCC\textit{mec} types were determined using different multiplex PCR assays identifying the \textit{ccr} gene complex (\textit{ccr} type), the \textit{mec} gene complex (\textit{mec} class), and the joining regions (subtype).\textsuperscript{29} VNTR was performed as previously described.\textsuperscript{12} The presence of the Panton-Valentine leukocidin (PVL) gene \textit{lukS} was determined by PCR as previously described.\textsuperscript{25}

**Decolonization of MRSA-positive humans**

Decolonization was performed according to clinical recommendations of the University Hospital of Bern.\textsuperscript{26} Mupirocin was applied thrice a day in both nares, a 0.1% chlorhexidine solution was gurgled twice daily to decolonize the pharynx, and the body and the hair were washed by showering with 4% chlorhexidine soap once a day. This procedure was repeated for 5 days. All clothes and bedding were changed and washed after the shower. Clearance of MRSA was assumed when two or more swabs were negative during the final follow-up screenings using a selective culture.\textsuperscript{26}

**Hygiene measures at the Equine Clinic**

Hygiene measures introduced at the Equine Clinic have been described in detail by Panchaud et al.\textsuperscript{21} Briefly, they consisted of using new gloves and hand disinfection (Sterillium\textsuperscript{8}; BODE Chemie, Hamburg, Germany) between contact with patients as well as stabling of equine MRSA-carriers in isolation facilities.

**Results**

**Occurrence and source of \textit{S. aureus} in horses**

A total of 70 clinical isolates of \textit{S. aureus} from hospitalized horses were isolated between January 2005 and January 2011. Among them, 12 isolates were methicillin-susceptible \textit{S. aureus} (MSSA) (17.1%), 18 were BORSA (25.7%), and 40 were MRSA (57.1%) (Fig. 1). Three additional MRSA were isolated from the nasal cavities of 3 of 135 horses screened on arrival during a 3-month period from January to March 2008. These three horses had already been hospitalized once at the clinic in the previous 6 months and had developed an MRSA infection at that time.

The 18 BORSA were isolated from the infection that developed after surgical procedures on 15 horses, thrombophlebitis after catheterization on two horses, and from the nasal discharge of one horse which developed rhinitis after hospitalization. The 40 MRSA originated from 34 horses that developed an infection after surgical procedures and from 6 with a thrombophlebitis after catheterization. The 12 MSSA were isolated from 7 horses that developed postsurgery wound infections and from 5 with thrombophlebitis. Each year, the percentage of postsurgical infections caused by MSSA was always lower (0%-4.7%) than the percentage of infections caused by multidrug-resistant \textit{S. aureus} (BORSA and MRSA) (6.1%-15.9%) (Table 1). Although the percentage of infections caused by BORSA decreased from 9.2% in 2006 to 0% in 2008, the percentage of infections caused by MRSA after surgical procedures increased from 0.9% in 2006 to 15.9% in 2010 (Table 1).

**Characteristics and occurrence of BORSA and MRSA**

Between January 2005 and August 2007, BORSA was responsible for a significant number of staphylococcal infections in horses (18/31, 58.1%) (Fig. 1). The majority of the BORSA isolates (16/18) shared the same VNTR profile and belonged to ST1 and spa type t2863 (ST1-t2863). The other BORSA isolates showed different VNTR profiles as BORSA ST1-t2863 and belonged to the clonal lineages ST1-t127 and to the new ST1660-t3043 (Table 2). They were only isolated once from infection sites in horses. All BORSA displayed the same multidrug-resistance profile with resistance to penicillin [\textit{blaZ}], to trimethoprim [\textit{dfr(A)}], to gentamicin and kanamycin [\textit{aac(6’)-Ie-aph(2’)-Ia}], to streptomycin [\textit{str}], and decreased susceptibility to oxacillin at an MIC of 1–2 mg/L. Only five BORSA isolates did not contain the streptomycin resistance gene \textit{str} including three BORSA ST1-t2863 as well as BORSA ST1-t127 and ST1660-t3043 (Table 2). During the outbreak with BORSA, four humans were also found to be carriers of the BORSA clone ST1-t2863 (Table 2 and Fig. 1).

The first MRSA infection in horses at the Equine Clinic of the University of Bern occurred in March 2006. This first MRSA isolate belonged to the clonal lineage ST398-t011-V (SCCM\textit{mec}), which was only detected at that time (Fig. 1). This unique MRSA ST398-t011-V isolate also differed from the other MRSA that has been causing infections since 2007 by the presence of the streptococcal resistance gene \textit{anti(6)-la}, the macrolide resistance genes \textit{msrA} and \textit{mph(C)}, and the tetracycline resistance gene \textit{tet(K)} (Table 2). Indeed, in February 2007, MRSA ST398-t011-IVA was detected in the nasal cavity of one member of the personnel (see below) and became rapidly responsible for most infections in horses caused by \textit{S. aureus}. Also, BORSA was not isolated at any time points after 2007 from infection sites (Fig. 1). Since then, all MRSA isolates from horses shared the same VNTR-profile and belonged to the same clonal lineage ST398-t011-IVA, except one isolated in October 2009 (ST398-t2970-IVA) and one isolated in January 2011 (ST8-t064-IVd) (Fig. 1 and Table 2). They all displayed resistance to \beta-lactams conferred by...
mecA and blaZ, to the aminoglycosides gentamicin and kanamycin \([\text{aac}(6')-\text{Ie}-\text{aph}(2')-\text{Ia}]\), to trimethoprim \([\text{dfr}(K)]\), and to tetracycline \([\text{tet}(M)]\), except one ST398-t011-IVa, which showed additional resistance to streptomycin \([\text{str}]\) and to macrolides and lincosamides \([\text{erm}(C)]\). This latter isolate was also present in the nasal cavity of one member of the personnel. Otherwise, the personnel was predominantly colonized with MRSA of the former ST398-t011-IVa lineage (Table 2 and Fig. 1). In October 2010, ciprofloxacin resistance emerged in one MRSA ST398-t011-IVa strain isolated from an infected wound of a horse after surgery. Ciprofloxacin-resistant MRSA ST398-t011-IVa was again isolated from infected sites of horses in November 2010 and January 2011. Apart from two MRSA isolates that displayed resistance to erythromycin and three to ciprofloxacin, all MRSA and BORSA isolates were susceptible to erythromycin and ciprofloxacin as well as to vancomycin, sulfamethoxazole, nitrofurantoin, linezolid, amikacin, chloramphenicol, and the combination quinupristin-dalfopristin. Neither BORSA nor MRSA from humans and horses contained the PVL gene.

**Evolution of BORSA and MRSA carriage in the personnel**

In 2006, 4 of 26 tested personnel carried BORSA ST1-t2863, and none carried MRSA. In February 2007, none of the 30 tested personnel carried BORSA, and 1 carried MRSA ST398-t011-IVa. In August 2007, 6 of 28 tested personnel carried MRSA ST398-t011-IVa, and none carried BORSA. In January 2008, 10 of 45 tested personnel carried MRSA ST398-t011-IVa (Table 1). One of them belonged to the anesthesia ward. In February 2008, MRSA carriers were decolonized at the University Hospital of Bern. Two of them were still positive after the third sampling in May 2008, when decolonization was stopped. Those two personnel were among the 6 of 33 tested persons that were MRSA-positive in 2010. They still harbored MRSA ST398-t011-IVa. This clonal lineage was also detected in one person who was previously decolonized and tested negative and in two people who never tested MRSA-positive earlier. In 2010, ST8-t064-IVd was detected for the first time among the personnel in Bern. It was detected in one newly hired person who started work at the clinic at the beginning of 2010. This newly hired person had worked earlier in a German horse clinic where this clone has been detected in both horses and humans\(^{39}\). One year later, MRSA ST8-t064-IVd was detected for the first time in the infection site of a horse at the Equine Clinic of Bern (Fig. 1). This MRSA ST8-t064-IVd isolate contained resistance genes conferring resistance to beta-lactams \([\text{mecA, blaZ}]\), trimethoprim \([\text{dfr}(A)]\), gentamicin and kanamycin \([\text{aac}(6')-\text{Ie}-\text{aph}(2')-\text{Ia}]\), and tetracycline \([\text{tet}(M)]\).
## Table 2. Genetic Characteristics and Antibiotic Resistance Profile of Borderline-Oxacillin-Resistant *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* Isolates from Horses and Humans

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin (n)</th>
<th>Genotype</th>
<th>Antibiotic resistance phenotype and genotype (resistance breakpoints in mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Horse</td>
<td>Human</td>
<td>VNTR</td>
</tr>
<tr>
<td>BORSA KM595-06</td>
<td>13</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>BORSA KM777-07</td>
<td>3</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>BORSA KM489-05</td>
<td>1</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>BORSA KM1549-2-06</td>
<td>1</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>MRSA KM1081-07</td>
<td>36*a</td>
<td>20</td>
<td>D</td>
</tr>
<tr>
<td>MRSA KM1219-09</td>
<td>1</td>
<td>0</td>
<td>E</td>
</tr>
<tr>
<td>MRSA KM1041-10</td>
<td>3</td>
<td>0</td>
<td>D</td>
</tr>
<tr>
<td>MRSA KM740-07</td>
<td>1</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td>MRSA KM344-06</td>
<td>1</td>
<td>0</td>
<td>D</td>
</tr>
<tr>
<td>MRSA KM1168-07</td>
<td>0</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td>MRSA SSi4</td>
<td>1</td>
<td>1</td>
<td>F</td>
</tr>
</tbody>
</table>

The MIC breakpoints determining resistance were those recommended for *S. aureus* in CLSI supplement M100-S20, except for streptomycin for which breakpoint from the French Society for Microbiology (www.sfm.asso.fr) was used.

*a* Three of them were identified in the nasal cavities of horses on admission into the clinic (screening in 2008).

*b* Strains KM346-08 and KM550-08 were susceptible to kanamycin with MIC <4 mg/L.

*c* Resistant to the tested drug, but the resistance mechanism was not determined.

MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; SCC*me*c, *Staphylococcus Cassette Chromosome mec*; VNTR, variable-number-tandem-repeat; TET, tetracycline; TMP, trimethoprim; STR, streptomycin; PEN, penicillin; OXA, oxacillin; KAN, kanamycin; GEN, gentamicin; ERY, Erythromycin; CLI, clindamycin; CIP, ciprofloxacin.
Decolonization of personnel and implementation of hygienic measures

The decolonization treatment was only applied to the personnel harboring MRSA. The decolonization of the personnel was performed 3 months after the introduction of hygienic precautions in January 2008. During the 6 months after decolonization, no horses developed an infection caused by MRSA, although two members of the personnel could not be decolonized and were still carriers (Fig. 1). During that time, only two postsurgical infections caused by MSSA were reported. Consequently, to decrease S. aureus infections, gloves were only worn for contact with horses with wounds. This had the unfortunate consequence of rapidly increasing the number of MRSA infections in horses in January 2009 (Fig. 1). In June 2009, the implementation of new glove rules was followed by mandatory hand-disinfection for each contact with patients as well as the clinic-owned horses. These measures limited the transmission of MRSA to horses but not to personnel. Indeed, from July 2009 to August 2010, the number of nosocomial infections in horses caused by MRSA and MSSA was limited to seven and one cases, respectively, though approximately one-fifth of the personnel (six people) were MRSA carriers (Fig. 1 and Table 1). From September 2010, laxity and lack of awareness in the application of hand hygiene were observed, which may be the cause of a re-increase of the S. aureus infections by the end of 2010 (Fig. 1).

Discussion

Between 2005 and 2010, two major clones of multidrug-resistant S. aureus caused nosocomial infections in horses. BORSA ST1-t2863, which was prevalent in wound infections until 2007, was rapidly replaced by MRSA ST398-t011-IVa. During this period, the personnel harbored the same strains in their nose as the ones causing infections in horses, indicating direct transmission between humans and horses. Indeed, the number of infections with BORSA in horses became less frequent once BORSA carriage in humans disappeared. Similarly, the number of infections with MRSA in horses and nasal carriage in the personnel increased rapidly after MRSA had been introduced into the Equine Clinic of Bern. Such a rapid spread of MRSA among the horses and personnel also happened in the Netherlands after the introduction of MRSA into the clinic. In Bern, MRSA ST398-t011-IVa was first detected among one member of the personnel; it has never been detected in horses earlier. This showed that the livestock-associated MRSA ST398-t011 is supported by the fact that this MRSA clone has also been successfully spreading in a variety of animal species as well as in humans during the past years.

Of note, the BORSA and MRSA strains of our study did not appear to be particularly more virulent than other S. aureus in horses. Infected equine patients did not suffer from increased frequency or severity of complications in wound healing, nor did colonized humans develop clinical signs. MRSA ST398-t011-IVa showed a high affinity for persistence and colonization and could not be eliminated from the nasal cavities of two members of the personnel using decolonization treatment. One year after decolonization, five people were MRSA carriers of the same MRSA clone ST398-t011-IVa, and one newly hired person carried MRSA ST8-t064-IVd. Thus, MRSA decolonization of equine clinic personnel is not very successful as long as the personnel are working in a clinical environment. This could be due to possible failures of the decolonization or to the presence of many risk factor such as antibiotic selective pressure and the spread of MRSA between horses and personnel. Additionally, repeated decolonization with mupirocin may select for a mupirocin-resistant MRSA-population. Persistent staphylococci carriage and intermittent staphylococci colonization in humans is well described and occurs in about 30% and 60% of the population, respectively. Intermittently, colonized people are colonized with different S. aureus strains at varying frequencies.

The use of antibiotics and the subsequent environmental contamination with antibiotics such as penicillin is an important risk factor for nasal colonization and transmission of antibiotic resistant bacteria to patients. Penicillin has been routinely used at the Equine Clinic of Bern for the prevention of infections before and after surgery, and the use of this antibiotic has been shown to select for specific multidrug-resistant staphylococcal flora including BORSA. To prevent the spread of multidrug-resistant S. aureus in the clinic, prophylactic use of penicillin has been limited and hygienic measures, including the changing of gloves between patients and the use of hand disinfectants, have been introduced. Horses infected with MRSA are isolated in special isolation stalls. Additionally, horses with a history of MRSA infection are currently directly isolated in case of hospitalization, because they have been shown to possibly still carry MRSA in their nasal cavity. The continuous application of these measures is necessary to limit the number of postsurgical wound infections and thrombophlebitis in horses caused by nosocomial multidrug-resistant S. aureus. Relaxation of hand hygiene measures led to a rapid increase in the number of MRSA infections in horses. Additionally, fluoroquinolones that have been used for the treatment of MRSA infections have likely selected for fluoroquinolone-resistant MRSA ST398-t011-IVa isolates. Therapeutic strategies also need to be established to limit the selection and the spread of such isolates.

This study showed that the livestock-associated MRSA ST398-t011 recently emerged in horses in Switzerland, presumably introduced into the clinic through the personnel. It rapidly replaced another multidrug-resistant S. aureus BORSA, which had been previously responsible for nosocomial infections at the Equine Clinic of Bern. Concurrent colonization of personnel with MRSA isolates indistin-
guishable from those recovered from horses has also been reported in other clinics. Our study supports the notion that humans play a role in the introduction and transmission of multidrug-resistant *S. aureus* within a clinic. It is, therefore, necessary to stop the transmission between personnel and patients using gloves and hand disinfectants, as hand are considered a major transmission vehicle for MRSA. Prevention of nasal carriage in humans needs to be addressed, as nasal decolonization was not successful as long as people were working in a clinical environment. Long-term surveillance as well as continuous and strict hygiene measures should be maintained to prevent the spread of nosocomial bacteria. Preventing nosocomial MRSA infections represents a new challenge to equine clinics requiring improved hygiene awareness among all personnel, beginning with strict hand sanitation.

**Acknowledgments**

We thank the personnel of the Equine Clinic who contributed to the sample collection, as well as the personnel of the Centre for Zoonoses, Bacterial Animal Diseases, and Antimicrobial Resistance (ZOBA). We are also grateful to Kathrin Mühlemann, Gudrun Overesch, Andrea Endimiani, and Andreas Thomann for advice. This study was partially financed by the installation grant of the University of Bern to V.P.

**Disclosure Statement**

No competing financial interests exist.

**References**


Address correspondence to:
Vincent Perreten, Ph.D.
Institute of Veterinary Bacteriology
University of Bern
Postfach
Laeggass-Strasse 122
Bern
Switzerland
E-mail: vincent.perreten@vbi.unibe.ch