Methicillin-resistant coagulase negative staphylococci isolated from horses

Marialaura Corrente, Maria D'Abramo, Francesca Latronico, Maria Fiorella Greco, Anna Lucia Bellacicco, Grazia Greco, Vito Martella, Domenico Buonavoglia

Department of Public Health, Faculty of Veterinary Medicine, University of Bari, Bari, Italy

SUMMARY

A methicillin-resistant (MR) *Staphylococcus epidermidis* strain was isolated from a saddle horse affected by osteolysis. MR coagulase-negative staphylococci (MRCNS) were isolated from 11 of 14 (78.8%) horses housed in the same riding club. By typing of the SCCmec region, almost the strains displayed a non typeable (NT) pattern and possessed the ccr type 2. Altogether, the high prevalence of MRCNS and the detection of NT SCCmec types support the hypothesis that horses may represent a reservoir of MRCNS for humans and that equine MRCNS may act as potential source of resistance genes for other staphylococci.

KEY WORDS: Methicillin-resistance, coagulase negative staphylococci, horses, humans

Received February 23, 2009 *Accepted* April 29, 2009

Methicillin-resistant *Staphylococcus aureus* (MRSA*)* and methicillin-resistant coagulase negative staphylococci (MRCNS) are an important cause of infections in humans (Deurenberg and Stobbering, 2008). Resistance to methicillin is regulated by staphylococcal cassette chromosome mec (SCC*mec*) element. Five major SCC*mec* variants (I to V) have been recognised, that appear to be variously distributed across the staphylococcal species (Milhereico *et al*., 2007), but there is evidence of non-typeable (NT) still unrecognised, SCC*mec* elements in some isolates (Qi *et al*., 2005). We detected a methicillin-resistant strain of *Staphylococcus epidermidis* (MRSE) in a horse with osteolysis. In addition, by screening the horses housed in the same shelter, MRCNS were identified that displayed NT SCC*mec* elements.

A 13-year-old saddle horse (71/05) was hospitalized at the Clinic of the Faculty of Veterinary Medicine in Bari, Italy. The animal presented lameness of the right forelimb. Radiographs showed osteolysis of the 3rd metacarpus that was surgically removed. After surgery the animal was treated with gentamycin and streptomycin for 7 days. From a fragment of the bone a strain of *S. epidermidis* was isolated exhibiting resistance to a wide spectrum of β-lactams. A PCR assay specific for the *mec*A gene (Murakami *et al.,* 1991) confirmed that the isolate was an MRSE. To evaluate the dissemination of methicillin-resistant staphylococci in the shelter, swabs with Amies transport medium were taken from the nares and the skin of the horse 71/05, from the nares of healthy horses (n=14) housed in the same riding club, from the nares of the personnel of the riding club (n= 5) and the horse owner. All the samples were inoculated on Mannitol Salt Agar (MSA, Oxoid, Milan, Italy) plus 6 µg/ml of oxacillin (Sigma, Milan, Italy). The staphylococci grown on MSA were biochemically identified with apiStaph (Biomérieux, Marcy L'Etoile, France) and tested by the *mec*A-specific PCR. Two MRCNS (*S. epidermidis* and *S. sciuri*) were isolated from the nares of horse 71/05 and 13 MRCNS of different species (Table 1) were isolated from 11 out of the 14 healthy horses (78.6 %). By means of disk diffusion test (Clinical and Laboratory Standards Institute, 2006) all the strains were

Corresponding author
Prof. Marialaura Corrente
Department of Public Health
Faculty of Veterinary Medicine
University of Bari, Bari, Italy
Str. prov. per Casamassima, km. 3
70010 Valenzano, BA, Italy
E-mail: m.corrente@veterinaria.uniba.it

found to be resistant to at least 2 classes of non β-lactam antimicrobials (Table 1). MIC_{90} of oxacillin (Etest, Ab biodisk, Solna, Sweden) was of 64 µg/ml. The virulence of all the strains was investigated by using PCRs targeting the *ica*A and IS256 genes (Gu *et al.,* 2005). Moreover, the isolates were subjected to multiplex PCR assays, for characterization of the SCC*mec* region, using previously described protocols (Milhereico *et al*., 2007).

TABLE 1 - *Isolation of MRCNS from saddle horses, antimicrobial susceptibility pattern, virulence genes and characterisation of the SCCmec cassette.*

Horse	*Source*	*Identification*	*mecA gene*	*MIC to oxacillin*[a]	*Resistotype*	*icaA*	*IS256*	*SCCmec type*	*ccrB type*
71/05 A	3rd metacarpus	*S. epidermidis*	+	4	E, Na, Pi, Sxt, Su	+	+	NT[b]	2
71/05 A	Right nare	*S. sciuri*	+	64	E, L, Na, Pi, Su	-	-	II	3
71/05 A	Left Nare	*S. epidermidis*	+	4	E, Na, Pi, Sxt, Su	+	+	NT	2
87/05 B	Nares	*S. conhii*	+	32	E, L, Na, Nor, Pi, Su, Te	-	-	II	3
87/05 C	Nares	*S. conhii*	+	32	E, L, Na, Nor, Pi, Su, Te	-	-	II	3
87/05 D	Nares	*S. saprophyticus*	+	4	E, Sxt, Su	-	-	NT	1
186/05 1	Nares	*S. lentus*	+	32	L, Te	-	-	NT	2
186/05 2	Nares	*S. lentus*	+	32	L, Na, Pi	-	-	NT	2
186/05 3	Right nare	*S. lentus*	+	32	L, Na, Pi	-	-	NT	2
186/05 3	Left Nare	*S. lentus*	+	32	L, Na, Pi	-	-	NT	2
186/05 4	Right nare	*S. lentus*	+	64	L, Na, Pi	-	-	NT	2
186/05 4	Left Nare	*S. lentus*	+	64	L, Na, Pi	-	-	NT	2
186/05 5	Nares	*S. epidermidis*	+	0.094	E, Na, Pi, Su	+	+	IV	3
186/05 6	Nares	*S. lentus*	+	64	Na, Pi, Nor	-	-	I	2
186/05 7	Nares	*S. sciuri*	+	64	Cl, E, Gn, L, Na, Pi, Su, Te	-	-	II	3
186/05 8	Nares	*S. lentus*	+	32	L, Na, Pi	-	-	NT	2
186/05 9	Nares	*Staphylococcus* spp.	-	nd[c]	nd	nd	nd	nd	nd
186/05 10	Nares	*Staphylococcus* spp.	-	nd	nd	nd	nd	nd	nd
186/05 11	Nares	*Staphylococcus* spp.	-	nd	nd	nd	nd	nd	nd

Antimicrobial abbreviations: Cl: clindamycin; E: erytromycin ; Gn: gentamycin; L: lincomycin; Na: Nalidixic acid; Nor: norfloxacin; Pi: pipemidic acid; Sxt: cotrimoxazole; Su: sulphametoxazole; Te: tetracycline. Other antimicrobials tested: chloramphenicol, ciprofloxacin, doxycycline, gentamicin, norfloxacin, teicoplanin, vancomycin. [a]µg/ml; [b]NT: Non Typeable with multiplex PCR for SCCmec cassette; [c]nd: not done.

The nasal and the tissue bone MRSE isolates of horse 71/05 were NT by the SCC*mec* PCR assays. Both the strains were characterised as *ccr* type 2 and possessed the virulence-associated *ica*A and IS256 genes. By PFGE of Sma-I digested chromosomal DNA (McDougal *et al.,* 2003), the two strains exhibited the same pulsotype, suggesting a clonal origin. The *S. sciuri* nasal isolate of horse 71/05 was characterised as SCC*mec* type II and *ccr* 3. The biological/genetic features of the isolates made from the healthy horses are summarized in Table 1. The majority of the strains (8 out 13) were found to be NT by the SCC*mec* PCR assays, showing two bands for the alleles A and C, while the predominant *ccr* element was of type 2. Only 1 strain (*S. epidermidis*, 186/05-5) possessed the virulence markers *ica*A and IS256. MRSA strains were not detected in the horses, and MR staphylococci were not isolated from the human nasal swabs.

Metacarpal osteolytic infection by MRSE occurred in a saddle horse and required surgical removal of the bone. The same strain was isolated from the nares of the animal, suggesting a generalized infection. Musculoskeletal and respiratory infections by *S. epidermidis* strains either resistant or susceptible to methicillin have been described in horses (Trostle *et al.,* 2001), while in humans MRSE is frequently responsible for bacteraemia (Miragaia *et al.,* 2002).

Multi-resistant MRSE strains may be susceptible to a very narrow spectrum of drugs (Miragaia *et al.,* 2002). In this case empirical therapy with gentamycin was successful, as confirmed by the *in vitro* susceptibility tests. In addition, the strain displayed invasive attitude, as revealed by the presence of the *ica*A and *IS256* genes. Such genetic elements regulate biofilm production and are more frequent in sepsis-associated coagulase-negative staphylococci rather than in saprophytic strains (Gu *et al.,* 2005).

The horse owner and the personnel of the riding club were not carriers of methicillin-resistant staphylococci. However, a high number of MRCNS was found in the healthy horses, suggesting adaptation of MRCNS to equine bacterial flora. Most of the strains were multi-resistant and were NT by PCR genotyping of the SCCmec region. Similar NT pattern (bands A-C) have been identified in MRCNS strains of equine origin in other studies (Baptiste *et al*., 2005). It is likely that, in addition to the five major SCC*mec* allelic types, other SCC*mec* elements, still unrecognized, exist in nature (Qi *et al*., 2005).

Importantly, most of the staphylococcal strains analyzed in this study displayed a *ccr* type 2. Such *ccr* allele was found in conjunction with SCC*mec* types I and II, and with either A-E or A-C non-typeable SCC*mec* patterns . The *ccr* gene complex encodes recombinases that are responsible for mobility of the SCC*mec* region. Unlike *ccr* types 1 and 3, *ccr* type 2 is highly permissive to genetic transfer of SCC*mec* gene (Qi *et al*., 2005). These findings raise concerns about the possibility that equine MRCNS may act as a reservoir of new resistance genes for other staphylococci, including *S. aureus*.

In conclusion, we identified equine MRCNS strains that harboured novel SCC*mec* elements and some of those MRCNS strains were potentially invasive. In several countries, including Italy, retired or injured riding horses are slaughtered and there is consumption of either slightly cooked or raw equine meat. Humans may be exposed to methicillin-resistant staphylococci of equine origin and this may also create the conditions for a flow of genetic material between animal and human and/or between non-pathogenic and virulent staphylococci. Accordingly, the study of drug-resistance in staphylococcal strains of animal origin is pivotal to understand the impact of MRSA and MRCNS on both animal and human health.

REFERENCES

Baptiste K.E., Williams K., Willams N.J., Wattret A., Clegg P.D., Dawson S., Corkill J.E., O'Neill T., Hart C.A. (2005). Methicillin-resistant Staphylococci in companion animals. *Emerging Infectious Diseases*. **11**, 1942-1944.

Clinical and Laboratory Standards Institute (2006). Performance standards for antimicrobial disk susceptibility tests. Approved standard. M2-A9 Villanova, Pa: Clinical and Laboratory Standards Institute.

Deurenberg R.H., Stobbering E.E. (2008). The evolution of *Staphylococcus aureus*. *Infection, Genetics and Evolution*. **8**, 747-763.

Gu J., Li H., Li M., Vuong C., Otto M., Wen Y., Gao Q. (2005). Bacterial insertion sequence IS256 as a potential molecular marker to discriminate invasive strains from commensal strains of *Staphylococcus*

epidermidis. *Journal of Hospital Infection*. **61**, 342-348.

McDougal L.K., Steward C.D., Killgore G.E., Chaitram J.M., McAllister S.K., Tenover F.C. (2003). Pulsed-Field Gel Electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *Journal of Clinical Microbiology*. **41**, 5113-5120.

Milhereico C., Oliveira D.C., de Lencastre H. (2007). Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. *Antimicrobial Agents and Chemoterapy*. **51**, 3374-3377.

Miragaia M., Couto I., Pereira S.F.F., Kristinsson K.J., Westh H., Jarløv J.O., Carriço J., Almeida J., Santos-Sanches I., de Lencastre H. (2002). Molecular characterization of Methicillin-resistant *Staphylococcus epidermidis* clones: evidence of geographic dissemination. *Journal of Clinical Microbiology*. **40**, 430-438.

Murakami K., Minamide W., Wada K., Nakamura E., Teraoka H., Watanabe S. (1991). Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *Journal of Clinical Microbiology*. **29**, 2240-2244.

Qi W., Ender M., O'Brien F., Imhof A., Ruef C., McCallum N., Berger Bächi B. (2005). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Zurich, Switzerland (2003): prevalence of Type IV SCC*mec* and a new SCC*mec* element associated with isolates from intravenous drug users. *Journal of Clinical Microbiology*. **43**, 5164-5170.

Trostle S.S., Peavey C.L., King D.S., Hartmann F.A. (2001). Treatment of methicillin-resistant *Staphylococcus epidermidis* infection following repair of an ulnar fracture and humeroradial joint luxation in a horse. *Journal of American Veterinary Medical Association*. **218**, 544-559.

Wu S., Piscitelli C., de Lencastre H., Tomasz A. (1996). Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of *mec*A from a methicillin susceptible strain of *Staphylococcus sciuri*. *Microbial Drug Resistance*. **2**, 435-441.