MRSA clone ST398-SCC*mec* IV as a cause of infections in an equine clinic

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ABSTRACT

Thirteen multidrug-resistant MRSA strains from infections of hospitalized horses in an equine clinic were analyzed. They all were indigestible by *Sma*I restriction, possessed SCC*mec* type IV and belonged to *spa* type t011. One isolate was analyzed by MLST and allotted to ST398. The MRSA clonal lineage ST398-SCC*mec* IV appears to have a high capability of causing clinical infections in an equine hospital environment.

INTRODUCTION

Staphylococcus aureus is an important pathogen, both for animals and for humans. Resistance of this bacterium to methicillin and all other beta-lactam antimicrobial agents has been a point of major concern for several decades, especially in human hospital settings, since the so-called methicillin-resistant *S. aureus* (MRSA) strains tend to accumulate resistance genes to most other antibiotics as well, making it difficult to treat infections with these strains. Certain epidemic clones of these MRSA strains are disseminating in and between hospitals, causing major outbreaks (Enright, 2003). In contrast to these hospital-acquired (HA) MRSA strains, community-acquired (CA) MRSA strains belong to different epidemic clones and are generally less resistant to antimicrobial agents other than β-lactams (Eady and Cove, 2003).

The presence of MRSA in animals had already been described in the early 70's (Devriese *et al.*, 1972), but more recently the importance of MRSA's in veterinary medicine has been increasing, especially as a cause of infection in intensive farm animals and as an occupational health hazard for people in contact with these animals (Voss *et al.*, 2005; Moodley *et al.*, 2006; Wulf *et al.*, 2006). Western European pigs appear to be highly colonized by a porcine-related clone, namely ST 398 (Voss *et al.*, 2005; Guardabassi *et al.*, 2007). In horses, MRSA has also been described as a problem. Outbreaks in equine clinics have been described in the USA (Seguin *et al.*, 1999) and Canada (Weese *et al.*, 2005), and several MRSA infections have been identified in Irish horses (O'Mahony *et al.*, 2005; Maeda *et al.*, 2007).

In a Belgian horse clinic it has been noted since the end of 2005 that MRSA was increasingly being isolated from clinical infections, often post-surgery. In view of the possibility of the presence of a nosocomial pathogen in this clinic, it was the aim of our study to examine a possible epidemiological relationship between the different MRSA isolates.

MATERIALS AND METHODS

Thirteen MRSA strains isolated from hospitalized patients over the period November 2005 through December 2006 were included in this study. These strains were associated with skin and wound infections (n = 8), foreign body infection (n = 1), peritonitis (n = 1), arthritis (n = 1) and respiratory tract infections (n = 2) (Table 1). A single isolate per horse was included. The horses all belonged to different owners and originated from France (n = 2) and from 6 different Belgian provinces (n = 11). They were of various breeds, gender and age. Between admission to the clinic and the isolation of MRSA, they were treated with various antimicrobial agents such as penicillin, gentamicin, neomycin, trimethoprim-sulfonamide combinations, ceftiofur and enrofloxacin. In several cases, failure of the therapy was the reason for submission of a sample for bacteriological analysis. *S. aureus* was identified using conventional

Table 1. Isolation sites of MRSA strains from horses in this study.

Strain n°	Lesion/sample from which the strain originated
10272	Peritoneal fluid from foal affected by peritonitis after explorative laparatomy
10773	Infection of incision site after abdominal surgery
06R314	Infection of incision site after abdominal surgery
06R1178	Infection of incision site after abdominal surgery
06R1335	Pacemaker infection
06R1415	Wound infection
06R1584	Tracheal sputum
06R1656	Tarsal joint infection
06R1700	Funiculitis after castration
06R1971	Wound infection after conservative wound treatment
06R1990	Abdominal wound
06R1995	Swab from guttural pouch
06R2062	Abscess within mastocytoma

phenotypic methods such as colony morphology on 5% sheep blood agar (Gibco, Paisley, Scotland), Dnase and acetoin production and growth on modified Baird-Parker medium (Devriese, 1981), as well as by PCR for detection of the *fem*A gene (Mehrotra *et al.*, 2000) and tDNA-intergenic spacer PCR (Baele *et al.*, 2000). Oxacillin resistance was phenotypically assessed by disk susceptibility testing on Isosensitest agar (Oxoid, Basingstoke, UK) at 30°C, and by MIC determination according to CLSI (2007), and additionally genotypically by detection of the *mec*A gene using the PCR method of Mehrotra *et al.* (2000). Production of beta-lactamase was assessed using beta-lactamase diagnostic tablets (Rosco, Taastrup, Denmark). Susceptibility was determined using MIC tests according to CLSI (2007) for lincomycin, erythromycin, tylosin, neomycin, gentamicin, tetracycline, enrofloxacin, trimethoprim and sulfonamides. For lincomycin, tylosin and neomycin, the resistance assessment was based on bimodal distribution by comparing the MIC values of the thirteen horse strains with the MICs of *S. aureus* strains from other origins. Furthermore, multiplex PCR reactions were performed as previously described for the following resistance genes: *ermA*, *ermC*, *tetK*, *tetM*, *aac6'-aph2"*, *ant4'* and *aph3'* (Denis *et al.*, 2000).

Genotyping of the strains was performed by Pulsed Field Gel Electrophoresis (PFGE) according to the protocol of Deplano *et al.* (2000), by SCC*mec* typing as described by Oliveira and de Lencastre (2002) and Zhang *et al.* (2005), and by *spa* typing (Harmsen *et al.*, 2003). Multilocus sequence typing (MLST) (Enright *et al.*, 2000) was carried out for one representative strain. PCRs for Panton Valentine Leucocidin (PVL), Toxic Shock Syndrome Toxin-1 (TSST-1) and exfoliative toxins A and B were also performed on all strains, as described previously (Lina *et al.*, 1999; Jarraud *et al.*, 2002).

RESULTS

The results are shown in Table 2. In summary, the strains were resistant to all beta-lactams. Ten strains additionally showed resistance to macrolides and lincosamides (ML) (erythromycin, tylosin and lincomycin) (through the *ermC* gene), to tetracycline (encoded by *tetM*), and to gentamicin. Resistance to neomycin was variable. The presence of aminoglycoside resistance genes varied between strains. There were three different MRSA strains, as they were susceptible to ML. It was furthermore typical that all strains showed resistance to trimethoprim and were sensitive to sulfonamides. All isolates produced beta-lactamase.

Genotyping was first attempted by PFGE, but the strains could not be digested with the SmaI restriction enzyme. SCC*mec* and *spa* typing revealed that all thirteen strains possessed SCC*mec* type IV and belonged to *spa* type t011. By MLST, one representative strain belonged to sequence type (ST) 398. The PCRs for PVL, TSST-1 and the exfoliative toxins, A and B, were negative.

DISCUSSION

All the isolates included in this study were non-typeable through PFGE using *Sma*I digestion, contained SCC*mec* element IV and belonged to *spa* type t011. This characterizes them as belonging to the ST398 clonal lineage (de Neeling, 2007; Witte *et al.*, 2007), which was confirmed in this study by MLST typing of one representative isolate. *S. aureus* strains belonging to the clonal lineage ST398 have long been considered rare (Witte *et al.*, 2007). They have been most extensively described as isolates from infections and from nasal colonization in pigs and in pig farmers (Armand-Lefevre *et al.*, 2005; de Neeling *et al.*, 2007; Guardabassi *et al.*, 2007). This clone is typically indigestible by the *Sma*I restriction enzyme due to the presence of a novel DNA methylation enzyme (Bens *et al.*, 2006). Voss *et al.* (2005) suggested that this MRSA strain was associated with pig farming and was able to spread not only from pigs to humans, but also between humans.

On the other hand, MRSA strains belonging to ST398 have been occasionally isolated in dogs and

Table 2. Results of genotyping and antimicrobial susceptibility testing of the thirteen horse MRSA strains included in this study.

Strain n°	PFGE *type	SCC *mec* type	*Spa* type type	MLST ST	Resistance in MIC test**	B-lactamase production	*mecA*	*ermA*	*ermC*	*tetK*	*tetM*	*aac6'-aph2"*	*ant4'*	*aph3'*
10272	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	-	-
10773	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	+	-
06R314	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	+	-
06R1178	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	+	-
06R1335	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	+	-
06R1415	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	+	-
06R1584	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	+	-
06R1656	nt	IV	t011		Oxa, Linco, Ery, Tyl, Neo, Gen, Tet, Trim	+	+	-	+	-	+	+	+	-
06R1700	nt	IV	t011		Oxa, Gen, Tet, Trim	+	+	-	-	-	+	+	-	-
06R1971	nt	IV	t011		Oxa, Neo, Gen, Tet, Trim	+	+	-	-	-	+	+	+	-
06R1990	nt	IV	t011		Oxa, Gen, Tet, Trim	+	+	-	-	-	+	+	-	-
06R1995	nt	IV	t011		Oxa, Linco, Ery, Tyl, Gen, Tet, Trim	+	+	-	+	-	+	+	-	-
06R2062	nt	IV	t011	398	Oxa, Linco, Ery, Tyl, Gen, Tet, Trim	+	+	-	+	-	+	-	-	-

* nt = not typable using *Sma*I digestion
** Oxa = oxacillin; Linco = lincomycin; Ery = erythromycin; Tyl = tylosin; Neo = neomycin; Gen = gentamicin; Tet = tetracyclin; Enro = enrofloxacin; Trim = trimethoprim; Sulfa = sulphachloropyridazin

horses (Witte *et al.*, 2007): the equine strains were isolated from sinusitis in a German foal and wound infections in two Austrian horses. Surprisingly, all thirteen strains that had been included in the present study and had been isolated from patient infections in the equine clinic over a period of more than one year, belonged to the clonal lineage ST398-SCC*mec* IV. Outbreaks in equine clinics have been described in the USA (Seguin *et al.*, 1999) and Canada (Weese *et al.*, 2005). However, in most veterinary hospital studies, the MRSA strains isolated from animals belonged to epidemic clones widely disseminated in human hospitals (Loeffler *et al.*, 2005; Weese *et al.*, 2005).

Since Voss *et al.* (2005) suggested that the "pig associated" clone ST398 can be transmitted from animals to humans and also between humans, it would be advisable to screen the persons in contact with hospitalized horses to examine them for carriership of this strain. In the event of a positive result, these people and their general practitioner would then be aware of the situation and could take measures, if necessary.

Furthermore, the fact that this strain has been isolated over a long time period in the equine clinic merits further investigation. *S. aureus* carriership by one or more of the clinic employees might have been responsible for recurrent transmission to the horse patients. High prevalence of MRSA strains has been reported among veterinarians and veterinary students, particularly those in contact with livestock (Hanselman *et al.*, 2006; Wulf *et al.*, 2006). In the study of Wulf *et al.* (2006), the MRSA strains isolated from these persons were also not digestible by SmaI and belonged to the related *spa* types t011, t034 and t108.

However, it is also not unlikely that the clone can persist in the clinical environment or on materials used in the clinic, since it has been described that *S. aureus* can persist in the dry environment from seven days up to seven months (Kramer *et al.*, 2006). In human MRSA isolates, a better environmental survival rate has been reported for outbreak as opposed to sporadic strains (Wagenvoort *et al.*, 2000).

As a third possibly important factor, the colonization rate of horses belonging to the healthy horse population with this clonal MRSA lineage also remains to be elucidated. It should be noted that the antimicrobial resistance patterns of the thirteen isolates included in this study were not completely identical, which points to the fact that several subtypes of this clone were implicated in the horses' infections. This may indicate that the horses might already have been carriers of the MRSA strain upon admission to the clinic. Research into this possibility is currently being carried out.

In conclusion, it can be stated that the MRSA clonal lineage ST398-SCC*mec* IV is capable of causing infections in an equine hospital environment, and that further research into the origin of this clone is needed.

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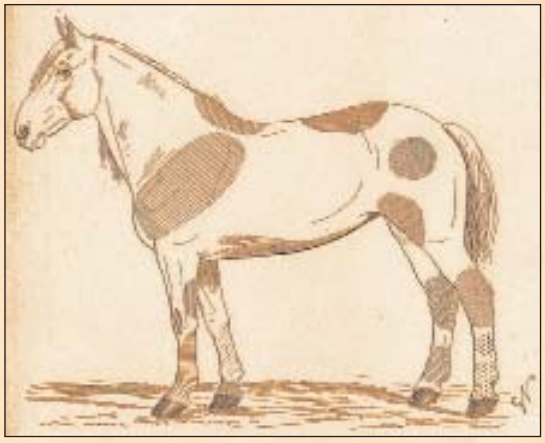
Uit het verleden

VAN VUURPUNT TOT ACUPUNCTUUR



Op de figuren: een 19de eeuwse voorstelling van de mogelijke vuurlijnen of vuurpunten bij het paard en een puntcauterisatieinset uit dezelfde tijd (*Schenking Van den Abbeele - Veterinaire Museumcollectie Merelbeke*). De set werkt als volgt. Een doorboorde metalen kogel wordt in het vuur verhit en met een aangepaste tang in de cauterisator geplaatst. Men steekt een naald doorheen de kogel die zodoende lange tijd verhit blijft. De diepte van de aan te brengen punten kan met schroefjes geregeld worden door de naald meer of minder ver doorheen de kogel te laten uitsteken en vervolgens op de gewenste diepte te fixeren.

Kunstmatig ontsteking opwekken was samen met 'aderlaten' de meest wijd verspreide diergeneeskundige ingreep vanaf de oudheid tot in de 19de eeuw. Men probeerde daarmee vooral chronische processen te 'activeren' of 'het kwaad' uit te laten. Zowel inwendige als uitwendige aandoeningen werden behandeld. Ook het profylactische gebruik was wijd verbreid bij dreigende epidemieën. Drie types ingrepen werden toegepast:

1. Oppervlakkige hittecauterisatie: vuurpunten en vuurlijnen zetten op de huid. Dit werd vooral bij paarden toegepast.

2. Kunstmatige ettervorming opwekken via het veroorzaken van zogenaamde fixatiefistels en fixatieabcessen (vooral bij runderen). Een irriterende stof werd onderhuids ingespoten of op een drager (drain, seton, metalen schijfjes) subcutaan ingebracht. De drager kon op zichzelf al een aanslepende 'vreemd voorwerp ontsteking' opwekken.

3. Acupunctuur werd oorspronkelijk in het confuciaanse China niet zozeer voor pijnbestrijding of anesthesie gebruikt. Dit was de meest milde vorm van ontsteking opwekken. De naalden werden soms verhit. Deze met puntcauterisatie verwante methode werd 'vuuracupunctuur' genoemd.

Luc Devriese