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Methicillin-resistant *Staphylococcus aureus* from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans

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ABSTRACT

A total of 272 methicillin-resistant *Staphylococcus aureus* (MRSA) from equine infections originating from 17 equine hospitals and 39 veterinary practices in Germany as well as 67 isolates from personnel working at equine clinics were subjected to molecular typing. The majority of isolates from horses was attributed to clonal complex (CC) 398 (82.7%). Within CC398, 66% of isolates belonged to a subpopulation (clade) of CC398, which is associated with equine clinics.

MRSA attributed to CC8 (ST254, t009, t036, SCC*mec*IV; ST8, t064, SCC*mec*IV) were less frequent (16.5%). Single isolates were attributed to ST1, CC22, ST130, and ST1660. The emergence of MRSA CC22 and ST130 in horses was not reported so far. Nasal MRSA colonization was found in 19.5% of veterinary personnel with occupational exposure to horses. The typing characteristics of these isolates corresponded to isolates from equine infections. Comparing typing characteristics of equine isolates with those of a substantial number of isolates from human infections typed at the German Reference Center for Staphylococci and Enterococci (2006–2014; n = 10864) yielded that the proportion of isolates exhibiting characteristics of MRSA from equine medicine is very low (<0.5%). As this low proportion was also found among MRSA originating from nasal screenings of human carriers not suffering from a staphylococcal infection (n = 5546) transmission of MRSA from equine clinics to the community seems to be rare so far.

1. Introduction

Staphylococcus aureus becomes methicillin resistant by acquisition of the *mec* genes (*mec*A and homologues) contained by staphylococcal cassette chromosome *mec* (SCC*mec*) elements from which at least 11 basic types are known so far. *S. aureus* shows a rather clonal population structure; typing of isolates by relevant methods reveals allocation to certain clonal types, in particular multilocus-sequence typing (MLST) and *spa*-typing are used as standard methodologies [1,2]. MRSA is globally prevalent in nosocomial settings as hospital-associated MRSA (HA-MRSA), which is mainly due to intra- and interhospital spread of epidemic clonal lineages [3–5]. In addition, MRSA emerged in the community without any relation to healthcare facilities (CA-MRSA, [6]). The first MRSA in animals was reported from cases of mastitis in dairy cattle in 1972, followed by sporadic observations of infections in various animals including postsurgical wound infections in horses [7]. Since 2006 MRSA attributed to clonal complex CC398 received specific attention since these so-called livestock-associated MRSA (LA-MRSA) is widely disseminated among various livestock animals mainly as an asymptomatic nasal colonizer [8,9]. Because of its capacity to cause a variety of infections in humans such as skin and soft-tissue infections, surgical wound and joint infections, invasive device infections (catheter, endoprostheses), ventilator-associated pneumonia, and septicemia [10–12] MRSA CC398 became a public health issue.

Furthermore, MRSA raised attention as nosocomial pathogens in companion animals and equine medicine. For companion animals, such as cats and dogs, clusters of MRSA infections in veterinary facilities were observed [13–15]. Several studies have provided evidence of hospital-associated (HA)-MRSA HA-MRSA transmission from humans to small animals in veterinary facilities and vice versa. Molecular typing

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of the isolates suggested an origin in human hospitals [16,17]. The first report of an outbreak of MRSA infections in horses in a veterinary hospital came from the United States in 1999 [18], and was followed by descriptions of clusters of MRSA infections in equine hospitals in Canada [19,20] and in Central Europe a few years later [21,22]. The majority of the Canadian MRSA isolates from horses and staff, as reported by previous studies, has typically been identified as Canadian epidemic MRSA-5, equivalent to "USA500", a putatively equine clinic associated strain, which accounted for nearly 10% of MRSA in Canadian hospitals by the end of the 1990 [23]. It exhibits MLST ST8, *spa* type t064 (corresponds to *spa* type 7 according to the Kreiswirth nomenclature), and contains SCC*mec*IV [20]. This strain type was also reported for MRSA isolates from horses from the United States and from Ireland [24,25]. In a Canadian veterinary hospital a cluster of skin and soft tissue infections in humans working there was also observed [26]. At this time the central European MRSA isolates from nosocomial infections in horses exhibited ST254, t036, and SCC*mec*IVh [21,22]. Meanwhile, MRSA CC398 is prevalent as a nosocomial pathogen in veterinary clinics, particularly in those for horses in Austria [22,27], Belgium [28,29], Germany [30], the Netherlands [31], Switzerland [32], and the United Kingdom [17]. Furthermore, nasal colonization of veterinary personnel attending horses was reported [22,31–33]. The majority of MRSA CC398 isolates from horse clinics exhibited a typical pattern of characteristics when subjected to typing: *spa* type t011, more rarely t6867, SCC*mec*IVa, and phenotypic resistance to gentamicin based on the *aac*A-*aph*D gene [22,31,32]. A more detailed analysis of the population-structure through mutation discovery at 97 loci revealed that MRSA CC398 from horse clinics exhibiting the above mentioned characteristics represent a particular subpopulation (clade) of LA-MRSA CC398 [34].

The significance of MRSA from colonization and infections in horses for infections in humans has not been assessed in more detail so far. Therefore, the objective of this study was to determine the proportion of typical equine MRSA clones among the MRSA from human infections based on a comparative analysis of typing characteristics.

2. Materials and methods

2.1. MRSA from infections in horses

The 272 isolates included originated from infections like soft-tissue and joint infections, pneumonia, sinusitis, metritis, omphalophlebitis or postoperative wound infections and were derived from horses treated in 17 veterinary hospitals as well as in 39 large animal practices in all regions of Germany (predominantly in the federal states of North Rhine-Westphalia and Lower Saxony) between January 2011 and February 2015.

2.2. MRSA from nasal swabs from veterinary personal and veterinarians ttending horses

We prospectively collected nasal swabs of employees (veterinarians and other staff; n = 349) in five equine clinics and three large practices from which also MRSA from infections in horses were derived between 2012 and 2015. This resulted in 67 MRSA isolates. Swabs were taken from both nostrils and processed as described previously [35].

2.3. MRSA CC398 from different types of human infections

Isolates included in the analysis comprised (i) a sample of MRSA isolates (n = 8912) which were sent to the German Reference Center for Staphylococci and Enterococci between 2006 and 2013 for strain characterization and typing in line with routine diagnostic procedures or in case of outbreak investigations., and (ii) MRSA isolates from blood cultures (n = 1952) which were prospectively (2011–2013) collected in North Rhine-Westphalia and *spa*-typed [36].

MRSA isolates from nasal swabs from humans: These isolates (n = 5546) originated from 150 different hospitals all over Germany. They were collected both from patients with no staphylococcal infection at admission to hospitals and from inpatients between 2006 and 2014 and sent to the German Reference Centre for Staphylococci and Enterococci for molecular typing.

Primary diagnostics, species identification of *S. aureus*, and further characterization by means of *spa*-typing, attribution to clonal lineages (complexes) and demonstration of *mec*A and of *mec*C as well as phenotypic antibiotic susceptibility testing were performed as described previously [35,37]. Minimum inhibitory concentrations (MICs) were determined for 18 antibiotics belonging to 15 antibiotic classes (including anti-staphylococcal β-lactams (penicillin, oxacillin), aminoglycosides (gentamicin), macrolides (erythromycin), lincosamides (clindamycin), tetracyclines (tetracycline), fluoroquinolones (ciprofloxacin, moxifloxacin), phosphonic acids (fosfomycin), glycopeptides (vancomycin,teicoplanin), oxazolidinones (linezolid), rifamycins (rifampicin), steroid antibiotics (fusidic acid) lipopeptides (daptomycin), glycylcyclines (tigecycline), pseudomonic acids (mupirocin), and cotrimoxazol). SCC*mec* elements were characterized by using a PCR approach including a combination of different PCRs as described (www.staphylococcus.net). PCR for *luk*-PV and for the genes of the immune evasion cluster (IEC) was performed as described previously [38]. PCR for IEC included *int*3 as a marker for integrase group 3 phages which usually contain the IEC. MRSA CC398 disseminated in equine clinics can be discriminated from LA-MRSA CC398 of other origin by means of a canonical SNPs (canSNPs [34]). By aligning whole genome sequences of three isolates of equine origin attributed to the equine clinic clade, one isolate of porcine origin (http://www.digibib.tu-bs.de/?docid=00058391) and 20 genomic sequences of isolates of different hosts attributed to CC398 published by Price et al. [39], we identified a further canSNP in position 1837869 in SAPIG1748 (AM990992.1,[40]), which can be easily identified by ordinary PCR and use of degenerated forward primers (SNP at the very 3′end, degenerated nucleotide in the second last position in bold): for the horse-specific clade of CC398, forward primer 1748 h1 5′ATGCTTTTTGGCCAGCT**I**T, (canSNP1748T), and for the general LA-MRSA CC398 subpopulation forward primer 1748u2 5′ ATGCTTTTTGGCCAGCT**I**G (canSNP1748G). As a reverse primer 1748r 5′ ATTACTCAAGGAACTGAA was used. PCR conditions (using PuReTaq Ready-To-Go PCR Beads (GE Healthcare)) were: 95 °C$^{5.00}$ [95 °C$^{0.30}$; 45 °C^{30}; 72 °C^{30}]x35; 72 °C$^{4.00}$. Correct amplification was confirmed by sequencing of the amplicons for reference strains SO385 (LA-MRSA subpopulation, [40]), and 71193 (human ancestral clade [41]), and 07-00334, (equine clinic subpopulation [34]).

This PCR was validated by application to a set of 195 isolates attributed to CC398 which was used for a phylogenetic analysis [34]. It comprised of 195 isolates from different host species (horse n = 53, human n = 80, pig n = 35, chicken n = 7, cattle n = 6, dog n = 5, turkey n = 4, goose n = 2, goat n = 1, cat n = 1, environment n = 1). PCR for antibiotic resistance genes and PCR conditions were performed as described previously [42]. For PCR detection of *dfr* genes we followed the protocols according to McDougal et al. [43] for *dfrA* and to Argudin et al. [44] for *dfr*G and *dfr*K.

3. Results

3.1. MRSA isolates from horses

As shown in Table 1 the majority of MRSA isolates from horses was attributed to CC398 (84.5%). Among the 135 isolates exhibiting *spa* type t011 and gentamicin resistance, n = 127 contained SCC*mec*IV and were attributed to the equine clinic specific clade by means of the canSNP in ORF1748. The latter also applied to 40 isolates exhibiting *spa* type t6867 and to isolates exhibiting the rare *spa* types t588, t779, t1255, t4628, t4872, t10643 and t13788.

Table 1
Characteristics of MRSA from equine infections in Germany (n = 272).

Number of isolates (%)	Typing characteristics			Clonal complex	Antibiotic resistance pattern[a]	Resistance genes
	Spa-type	SCC*mec*	canSNP			
123 (45.2%)	t011	IV	1748T	CC398	PEN, OXA, GEN, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)[a]
4 (1.47%)		IV		CC398	PEN, OXA, GEN, TET, SXT, CIP, MFL	mecA, *aac*A-*aph*D, *tet*(M)[a],*dfr*(K)
1 (0.37%)	t588	IV	1748T	CC398	PEN,OXA,GEN,TET,CIP,MFL	*mec*A, *aac*A-*aph*D, *tet*(M)
3 (1.1%)	t779	IV	1748T	CC398	PEN,OXA, GEN, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)
2 (0.74%)	t1255	IV	1748T	CC398	PEN, OXA, GEN, TET, CIP	*mec*A, *aac*A-*aph*D, *tet*(M)
1 (0.37%)	t2576	IV	1748T	CC398	PEN, OXA, GEN, ERY, CLI, TET, SXT	*mec*A, *aac*A-*aph*D, *ermA, tet*(M),*dfr*(G)
1 (0.37%)	t4628	IV	1748T	CC398	PEN,OXA,GEN,TET,CIP,MFL	*mec*A, *aac*A-*aph*D, *tet*(M)[a]
2 (0.74%)	t4872	IV	1748T	CC398	PEN, OXA, GEN, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)[a]
4 (1.47%)	t4872	IV	1748T	CC398	PEN, OXA, GEN, ERY, CLI, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)[a], *erm*C
36 (13.1%)	t6867	IV	1748T	CC398	PEN, OXA, GEN, TET	*mec*A, *aac*A-*aph*D, *tet*(M)[a]
2 (0.74%)	t6867	IV	1748T	CC398	PEN, OXA, GEN, TET, SXT	*mec*A, *aac*A-*aph*D, *tet*(M)[a],*dfr*(K)
2 (0.74%)	t6867	IV	1748T	CC398	PEN, OXA, GEN, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)
1 (0.37%)	t10643	IV	1748T	CC398	PEN, OXA, GEN, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)
1 (0.37%)	t13788	IV	1748T	CC398	PEN,OXA, GEN, TET,	*mec*A, *aac*A-*aph*D, *tet*(M)
183 (66.5%)	**Subtotal**					MRSA CC98, SNP1748T
8 (2.9%)	t011	V	1748G	CC398	PEN, OXA, GEN, TET	*mec*A, *aac*A-*aph*D, *tet*(M)[a]
1 (0.37%)	t1451	V	1748G	CC398	PEN, OXA, TET	*mec*A, *tet*(M)
1 (0.74%)	t779	II	1748G	CC398	PEN,OXA,,ERY,CLI,TET,	*mec*A, *tet*(M), *erm*C
21 (7.7%)	t034	V	1748G	CC398	PEN, OXA, ERY CLI, TET, CIP, MFL	*mec*A, *erm*A, *aac*A-*aph*D, *tet*(M)
12 (4.4%)	t034	V	1748G	CC398	PEN, OXA, ERY CLI, TET, CIP, MFL	*mec*A, *erm*C, *aac*A-*aph*D, *tet*(M)
1 (0.37%)	t693	V	1748G	CC398	PEN, OXA, TET	*mec*A, *tet*(M)
1 (0.37%)	t1292	IV		CC22	PEN, OXA, ERY, CLI, CIP, MFL	*mec*A, *erm*C
1 (0.37%)	t747	IV		CC22	PEN, OXA, ERY, CLI, CIP, MFL	*mec*A, *erm*C
1 (0.37%)	t549[b]			ST1660[c]	PEN, OXA, GEN	*mec*A, *aac*A-*aph*D
1 (0.37%)	t843	XI		CC130	PEN, OXA	*mec*C
1 (0.37%)	t127	IV		CC1	PEN, OXA	*mec*A
28 (10.3%)	t009	IV		CC8	PEN, OXA, GEN, ERY, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)
1 (0.37%)	t036	IV		CC8	PEN, OXA, GEN, ERY, CLI	*mec*A, *aac*A-*aph*D, *erm*A
3 (1.1%)	t064	IV		CC8	PEN, OXA, GEN, ERY, CLI, TET, SXT	*mec*A, *aac*A-*aph*D, *erm*C, *tet*(M),*dfr*(A)
3(1.1%)	t064	IV		CC8	PEN, OXA, GEN, TET, SXT, RAM	*mec*A, *aac*A-*aph*D, *tet*(M),*dfr*(A)
5 (1.8%)	t051	IV		CC8	PEN, OXA, CIP, MFL	*mec*A
272 (100%)	**Total**					

[a] PEN (penicillin G), OXA (oxacillin), GEN (gentamicin), TET (tetracycline), SXT (trimethoprim/sulfamethoxazole), CIP (ciprofloxacin), MFL (moxifloxacin), ERY (erythromycin), CLI (clindamycin), RAM (rifampicin). SNP1748T corresponds to the CC398 horse-clade specific SNP and SNP1748G corresponds to the CC398 general-clade specific SNP (see text for details).
[b] SCC*mec* was untypeable for this isolate.
[c] No clonal complex defined so far.

The other 44 CC398 isolates were PCR positive for canSNP1748G; 43 of them contained SCC*mec*V. This also applied to 8 isolates which exhibited *spa* type t011 and gentamicin resistance. One isolate exhibited *spa* type 779 and contained SCC*mec*II. The second most frequent clonal complex was CC8: 29 (11%) of the isolates were attributed to ST254 and 11 to ST8 (confirmation by MLST); all of these CC8 MRSA contained SCC*mec*IV.

Other clonal complexes were more rarely represented by two isolates attributed to CC22, one isolate to CC130 containing *mec*C, and one isolate to CC1. One further isolate was attributed to ST1660.

The resistance profiles also are shown in Table 1. Gentamicin resistance is strikingly frequent (85%) and mainly associated with isolates of the equine clinic associated clade. The majority of the isolates from horses were resistant to tetracycline (97.5 %) and to gentamicin (85%). Resistance to fluoroquinolones was found in 79% of the isolates of the equine clinic associated clade and in 77.5% of all isolates from horses. Furthermore, 15.6% were resistant to erythromycin, 14.7% to cotrimoxazole, and 1.1% resistant to rifampicin. Resistance to other antibiotics that are important for treatment of staphylococcal infections such as glycopeptides, linezolid, daptomycin, tigecycline as well as

Table 2
Characteristics of MRSA from nasal colonization of veterinarians and veterinary personnel.

Veterinary hospitals and veterinary practices	Proportion of MRSA colonization	CC398							CC8	
		Equine clinic clade, SNP 1748T				Non equine clinic LA-MRSA CC398, SNP 1748G				
		spa t011, SCC*mec* IV, GEN	*spa* t6867, SCC*mec* IV, GEN	*spa* t4628, SCC*mec*IV, GEN	*spa* t7829, SCC*mec*IV, GEN	*spa* t011, SCC*mec*V	*spa* t034, SCC*mec*V	*spa* t1255, SCC*mec*V	t009	t036
Equine clinic A,2012/2013	3/31 (10%)	0	0	0	0	0	0	0	3	0
Equine clinic B,2013	I: 4/50 (8%)	2	1	0	0	0	1	0	0	0
2014	II: 23/63 (36.5%)	5	6	1	1	7	2	1	0	0
Equine clinic C,2013	I: 4/48 (4.3%)	2	1	0	0	0	1	0	0	0
2014	II: 7/20 (35%)	4	1	0	0	1	1	0	0	0
Equine clinic D,2012	4/18 (22%)	2	0	0	0	0	0	0	0	2
Equine clinic E,2014	8/37 (21.6%)	2	6	0	0	0	0	0	0	0
Veterinary practice A	1/12 (3.3%)	0	0	0	0	1	0	0	0	0
Veterinary practice B	8/50 (16%)	4	0	0	0	1	3	0	0	0
Veterinary practice C	5/20 (25%)	1	2	0	0	1	1	0	0	0
Total	**67/349 (19.2%)**	**22**	**17**	**1**	**1**	**11**	**9**	**1**	**3**	**2**

Legend: GEN, gentamicin resistance; SNP1748T corresponds to the CC398 horse-clade specific SNP and SNP1748G corresponds to the CC398 general-clade specific SNP (see text for details).

fusidic acid and fosfomycin was not observed. All of the isolates were susceptible to mupirocin.

3.2. MRSA from nasal colonization of veterinary personnel and veterinarians

These data are shown in Table 2. Overall, 19.2% of 349 persons were colonized with MRSA. The characteristics of these isolates largely correspond to those from infections in horses.

3.3. MRSA isolates from infections humans

Among 10864 MRSA isolates from different types of infections in humans 195 were attributed to CC398 based on *spa*-typing. Of these 158 isolates exhibited *spa*-types which have been observed in CC398 isolates from horses, (t011: n = 88; t034: n = 51, t6867: n = 6, t1451: n = 5, t1255: n = 3, t2576: n = 3; t588: n = 1; t4628: n = 1). All of these 158 isolates were subjected to PCR for the can SNP in SAPIG1748; 143 isolates were attributed to non-equine LA-MRSA CC398, and 15 isolates were attributed to the equine clinic subpopulation. Of the latter ones 4 originated from blood cultutres. The characteristics of these isolates are shown in Table 3. The proportion of the equine clinic subpopulation among all of the 10864 isolates from infections in humans was 0.14%.

The characteristics of isolates attributed to CC8 exhibiting *spa*-types t009 (corresponding to ST254) and t064 (corresponding to ST8) are also shown in Table 1. Their proportion among all of MRSA from infections in humans was 0.07%.

Taken together MRSA with characteristics typical for horse clinics represented 0.21% of all MRSA from humans included in this study.

At first glance, the low proportion of MRSA CC398 attributed to the equine clinic specific clade might be interpreted as low virulence for humans. Therefore, we investigated the proportion of the equine clinic specific clade among isolates obtained from nasal screenings. Among 5546 isolates merely 342 were attributed to CC398 (6.1%). From these isolates 28 exhibited *spa* type t011 and resistance to gentamicin and only two of these isolates contained *SCCmec*IV and canSNP1748T. Furthermore, four isolates exhibited *spa* type t6867, contained SCC*mec*IV and canSNP1748T. This resulted in a proportion of 0.1% of the equine clinic associated clade among the 5546 isolates. There was only one isolate exhibiting *spa* type t009 and one further exhibiting *spa* type t064.

3.4. Demonstration of the immune evasion gene cluster (IEC) in isolates of horse and human origin attributed to CC8 and exhibiting the same spa-types

S. aureus of animal origin usually lacks the immune evasion gene cluster IEC [8,45]. It is contained by prophages of the i*nt*3 group and consists of an enterotoxin gene (*sea* or *sep*) and the basic immune evasion genes *sak* (staphylokinase), *scn* (staphylococcal complement inhibitory protein), and *chp* (chemotaxis inhibitory protein). Among isolates from horses attributed to CC398 (*spa* type t011), exhibiting gentamicin resistance and containing SCC*mec*IV, 10% of the isolates contained the IEC [38]. The isolates from infections in humans reported here, which were attributed to the horse-specific clade were all negative for the IEC. As shown in Table 4 isolates with *spa* type t009 from horses and from nasal swabs of veterinary personnel and veterinarians lack the IEC, whereas the two isolates from infections in humans contained it. Among isolates exhibiting *spa* type t064, IEC with the same pattern of immune evasion genes is contained by isolates of both horse and human origin.

4. Discussion

The data reported in this study confirm that MRSA CC398 has become the most frequent MRSA in veterinary hospitals caring for horses in Germany [30]. Based on a substantial number of isolates we specify that the majority of them were attributed to the equine clinic specific clade of MRSA CC398. Isolates exhibiting t011 are still prevalent among the equine clinic associated clade, this *spa* type was also exhibited by the early isolates of this clade as reported previously [34]. It is very likely that *spa* types t588, t779, t1255, t1451, t4628, and t4872 derived from *spa* type t011, because they differ by one genetic event (deletion of one repeat or one point mutation in a repeat) only (www.ridom.com). Also isolates exhibiting *spa* type t6867, which differs from t011 by several genetic events, evolved from isolates exhibiting t011 [34]. They represent the second most frequent *spa* type among isolates attributed to the equine clinic specific clade. A high prevalence of MRSA CC398, t6867 was also reported by a recent survey on MRSA among horses in Germany [30]. We also observed MRSA CC398 with *spa* types t011 and t034 which were not attributed to the equine clinic specific clade among both isolates from infections in horses and from colonization of veterinary personnel. These *spa* types are particularly frequent among MRSA CC398 from livestock. We hypothesize that the horses acquired them from veterinarians also attending livestock or via environmental transmission. The finding of isolates not attributed to the equine clinic associated LA-MRSA and exhibiting both *spa* type t011 and resistance to gentamicin underlines that *spa* typing for the epidemiological analysis of MRSA infections in horses has to be supplemented by additional genomic markers.

Among all horse isolates about 15% were attributed to CC8 and 13% exhibited *spa* types that were already described for MRSA from horse clinics in Northern America and in Europe such as t064, t036 and t009 [14,22,46]. MRSA with *spa* type t051, SCC*mec*IV have not been reported from horses so far and the isolate detected in our study was attributed to ST8 by MLST. This is in contrast to MRSA t051 of human origin which is usually attributed to ST247 and contains SCC*mec*I [3–5]. *Spa*-type t051

Table 3
Proportion of MRSA from infections in humans (n = 10,864) which were attributed to CC398 and CC8 exhibited typing characteristics as MRSA which were typically associated with infections in horses.

Typing characteristics			Clonal complex	Antibiotic resistance pattern	Relevant resistance genes	Number of isolates	Proportion
spa-type	SCC*mec*	canSNP					
t011	IV	1748T	CC398	PEN, OXA, GEN, TET	*mec*A, *aac*A-*aph*D, *tet*(M)	5	0.046%
t588	IV	1748T	CC398	PEN, OXA, GEN, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *tet*(M)	1	0.009%
t1255	IV	1748T	CC398	PEN, OXA, GEN, TET,CIP,MFL	*mec*A, *aac*A-*aph*D, *tet*(M)	2	0.018%
t4628	IV	1748T	CC398	PEN, OXA, GEN, ERY, CLI, TET	*mec*A, *aac*A-*aph*D, *tet*(M), *erm*(C)	1	0.009%
t6867	IV	1748T	CC398	PEN,OXA,GEN,TET	*mec*A, *aac*A-*aph*D, *tet*(M)	6	0.055%
Subtotal CC398						15	0.137%
t009			CC8 (ST254)	PEN, OXA, GEN, ERY, CLI, TET, CIP, MFL	*mec*A, *aac*A-*aph*D, *erm*(A), *tet*(M)	2	0.018%
t064			CC8 (ST8)	PEN, OXA, GEN, TET, CIP, SXT	*mec*A, *aac*A-*aph*D, *erm*(C), *tet*(M), *dfr*(A)	6	0.055%
Subtotal other CC						8	0.074%
Total						**23**	**0.212%**

PEN (penicillin G), OXA (oxacillin), GEN (gentamicin), TET (tetracycline), SXT (trimethoprim/sulfamethoxazole), CIP (ciprofloxacin), MFL(moxifloxacin), ERY (erythromycin), CLI (clindamycin), DAP (daptomycin).

Table 4
Occurrence of the immune evasion gene cluster in MRSA isolates attributed to clonal complex CC8 from horses and humans.

Origin	Number of isolates	*Spa*-type	PCR for *int*3	PCR demonstration of IEC genes				
				sea	*sep*	*sak*	*chp*	*scn*
Infection in horses	28	t009	−	−	−	−	−	−
	6	t064	+	+	−	+	+	+
Nasal colonization of veterinarians and other personnel	3	t009	−	−	−	−	−	−
Infections in humans	1	t009	+	+	−	+	+	+
	1	t009	+	+	−	+	−	+
	6	t064	+	+	−	+	−	+

IEC = immune evasion cluster.

differs from t008 which is most frequent among isolates attributed to ST8 by deletion of only one repeat (www.ridom.com).

The results of our study indicate that spread of MRSA from equine clinics to the community is rare so far. Two main routes of MRSA spread from veterinary hospitals into the human community are possible: (i) Colonization of veterinarians. Indeed, earlier studies observed that the prevalence of MRSA colonization in families of livestock veterinarians was 9.5% [35]. (ii) Transmission of MRSA from animals that are still positive when discharged from the hospital to their caretakers "at home" and further dissemination from thereon in human and animal populations as suggested by an observation in Belgium [33].

Frequencies of colonization of humans with occupational exposure in horse clinics in outbreak situations reported by other studies were 13.7% for an Austrian university veterinary hospital [22], 9.4% for a Dutch veterinary hospital [31] and up to 22.2% for a Swiss university veterinary hospital [32]. According to our recent data nasal colonization of humans in veterinary hospitals attending horses is still high and above the proportion reported for veterinarians and veterinary personnel in general [47,48] besides livestock veterinarians [35]. The typing characteristics of the isolates from veterinarians in this study mostly reflect the clonal lineages of MRSA from equine infections. Of particular interest is the demonstration of LA-MRSA CC398 not attributed to the equine clinic specific clade, which might have been acquired by the veterinarians whilst working with livestock animals. The capacity of LA-MRSA CC398 for causing infections in humans is well documented (for summary see [10–12]). Although the proportion of LA-MRSA among all MRSA from infections in humans is comparatively low in Germany [10,11,49], it amounts up to 14% in hospitals which are located in German geographical areas with a high density of conventional animal farming facilities [11]. As reported here, the proportion of MRSA attributed to the equine clinic specific subpopulation of MRSA CC398 among all MRSA from infections in humans seems to be very low so far. We hypothesize that this is rather due to limited dissemination beyond humans exposed to horses with MRSA colonization and/or infections than to low virulence for humans. This is also suggested by finding isolates attributed to the equine clinic specific clade among blood culture isolates. Among the MRSA from nasal swabs taken at admission to hospitals from asymptomatic carriers we found the same low proportion. It remains to be shown whether the equine clinic specific clade of CC398 has a lower virulence potential for humans than MRSA CC398 from livestock. MRSA attributed to CC8 and exhibiting gentamicin resistance and *spa* type t009 or t064 were very rare among MRSA isolates from infections in humans in Germany. MRSA from horses with *spa* type t009 lacked the immune evasion gene cluster as previously shown [14]. We found the same for isolates from nasal colonization of veterinarians. Isolates from infections in humans, however, contained it.

MRSA with *spa* type t009 were widely disseminated in Northern German hospitals in the 1990s ("Hannover area epidemic MRSA" [5]) and became rare thenceforth. Thus, MRSA with *spa* type t009 from infections in humans are very likely remainders of this previously epidemic HA-MRSA. We should, however, be careful with deducing recent mutual exchange between human and veterinary hospitals simply from the presence of the IEC in horse isolates. We found IEC also in an MRSA isolate ST8, SCC*mec*IV, t064 that originated from an Austrian veterinarian hospital in 2007. Low proportions of MRSA CC8, *spa* type t064, SCC*mec*IV among all MRSA from infections in humans was also reported from a medical center in Chicago (0.7% [50]), whereas it was slightly higher among sporadic MRSA isolates from Ireland (2 %, [51]) and amounted to 6% among isolates from three hospitals in New York [52]. Fortunately, there are only a few published reports on infections with equine MRSA clones in veterinarians and veterinary personnel so far such as on a cluster of infections with MRSA CC8, spa type t064, SCC*mec*IV in Canada [20].

A few equine isolates (1.85%) were attributed to clonal lineages ST1, ST22, ST130 and ST1660. MRSA ST1, *spa* type t127 were already reported from equine infections in Austria in 2007 [22] where they are still observed [27]. They were rare in Germany among CA-MRSA, which contain *luk*-PV [42]. In Romania however, MRSA ST1 is prevalent among the MRSA from nosocomial infections in humans [53]. The occurrence of MRSA isolates with *spa* types associated with ST22 recently increased among MRSA from nosocomial infections making ST22 the most expanding MRSA clone in Europe [54]. MRSA attributed to CC130, usually containing *mec*C, are widely disseminated among animals, in particular ruminants [55]. In this study, we report the first infection with MRSA CC130 containing *mec*C in a horse. For MRSA CC130 from infections in humans a zoonotic origin is rather likely [56]. They are, however, rare among the MRSA from human infections in Germany [37]. It seems that MRSA belonging to ST1660 are also specific for equine clinics. They were already reported from a Swiss horse hospital [32] and putatively represent a horse associated strain. However, this clonal lineage is not disseminated in other animals or prevalent among infections in humans.

Finally, the high proportion of isolates that were resistant to fluoroquinolones requires specific attention. Resistance to these antibiotics was obviously infrequent or absent among isolates from earlier studies in Europe [22,32,29] and Canada [20]. Its rise during the last years probably reflects selective pressure by use of fluoroquinolones in equine clinics. For dissemination of MRSA in medical hospitals use of ciprofloxacin is known as risk factor for dissemination of epidemic HA-MRSA resistant to these antibiotic [56].

Taken together, MRSA from infections in horses mostly seem not to spread beyond equine medicine and therefore are rare among the MRSA from infections in humans. However, as MRSA from infections in humans in Germany are not notifiable besides those of the bloodstream and central nervous system, we cannot exclude the possibility of infections in humans with occupational or other contacts to horses with MRSA colonization and infections. In case of infections there are still sufficient alternatives for antibiotic treatment.

5. Conclusions

This study shows that MRSA infections in horse clinics are a nosocomial problem and that there is mutual transmission between humans and horses. Therefore surveillance at the interface of human and veterinary medicine is warranted. This should also pay attention to the apparent international dissemination of different clonal lineages of MRSA in horses and to the emergence of human hospital-associated epidemic MRSA in equine clinics. There is an urgent need for establishing and implementing guidelines on the prevention and control of MRSA spread among horses.

Ethical disclosure

Concerning sample collection the study protocol and data handling the study were approved by ethical committee of the Otto von Guericke University Magdeburg, affiliated to the faculty of medicine (file #47/09).

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