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Beyond MLST, PFGE, SCC*mec*- and *spa*-typing:

Comparative analysis of MRSA strains of equine and human origin

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Introduction

Staphylococcus aureus is a commensal as well as an important pathogen in human and veterinary medicine (8, 9). Based on previous studies, some animal *S. aureus* lineages appeared to be host-specific, in particular bovine- and ovine-specific lineages (7).

Since methicillin-resistant isolates of *S. aureus* (MRSA) are increasingly reported as an emerging problem in horses, pets and livestock animals, and therefore, questions about distinct species specific adaptation of certain genotypes have risen.

Currently, the epidemiology of MRSA in horses is poorly understood, and there also is a lack of information regarding virulence and resistance determinants associated with MRSA strains from clinical specimens of equine patients.

Therefore, the aim of this study was to provide a broad spectrum of genetic characteristics of equine MRSA strains to allow comparative analysis of the study collection as well as to assess the relationship to epidemic human strains by means of a large number of molecular typing results beyond MLST, PVL, SCC*mec*- and *spa*-typing.

Conclusion

Extensive molecular typing of MRSA from equine and human origin revealed that all strains investigated share similar genetic backgrounds. Minor differences (slv of MLST genes, variations in SCC*mec* composition) were presumably incidental. Some differences in other factors (i. e. *sak*, *chp*, *scn)* might be related to differences in the pathophysiology of different host species.

The data presented here allow a broad insight into MRSA strains belonging to genetic lineages CC8 (ST8/ST254) and CC22 (ST22/ST1117).

The minimal differences detectable between strains adapted to human and equine hosts may provide evidence for the possible easiness of interspecies transferability of certain MRSA genotypes: Strains belonging to these lineages seem to be adapted to more than one mammalian species, and we therefore propose to designate these genotypes "extended host spectrum genotypes (EHSG)".

Comparative analysis of 19 equine and representative human MRSA strains

Tab.1: Resistance and virulence genes

Strain ID	Number	Host	MLST[1]	Clonal Complex	PFGE-Typ[2]	spa-Typ[3]	Methicillin resistance and SCCmec-associated genes													B-lactamase		Macrolide/Lincosamide/Streptogramin resistance genes					Aminoglycoside resistance genes		
							mecA	delta mecR	mecI/R, xylR	ugpQ	ccrA/B-1	plsSCC-COL	Q9XB68-dcs	ccrA/B-2	kdpa to kdpE	ccrA/B-3	merA/B/R/T	ccrAA/C	ccrA/B-4	blaZ	blaI/R	ermA	ermC	linA	msrA/mefA/mphBM	vat genes,vga/b	aacA-aphD	aadD	aphA3
North German E-MRSA (134/93)	1	Human	247	8	EP[4]	t051	x	x		x	x	x	x							x	x	x					x	x	
IMT-6634; -8142	2	Horse	8	8	IMT-H/-H-1	t064	x	x		x			x	x						x	x		x				x		
IMT-10697	1	Horse	8	8	IMT-H	t064	x	x		x			v	x						x	x						x		
Hannover E-MRSA (1000/93)	1	Human	254	8	EP[4]	t009	x	x		x			x	x			x	x		x	x	x					x		x
Hannover E-MRSA (clinical isolates)	5	Human	254	8	n.t.	n.t.	x	x		x			x	v			v	v		x	x						x		x
IMT-11900; -11901; -8940	3	Horse	254	8	IMT-G	t009	x	x		x			v				x			x	x						x		x
IMT-8936	1	Horse	254	8	IMT-G	t009	x	x		x			x														x		x
IMT-10029; -8591; -6638; -6637; -9658; -11918	6	Horse	254	8	IMT-G/-G-1	t036	x	x		x			x				x			x	x						x		x
IMT-6636	1	Horse	254	8	IMT-G	t036	x	x		x			x														x		x
IMT-12562	1	Horse	254	8	IMT-G	t036	x	x		x			v				x			x	x						x	x	x
IMT-9762	1	Horse	254	8	IMT-G	t036	x	x		x			x				x			x	x						x		x
Vienna E-MRSA (635/93)	1	Human	239	8	EP[4]	t037	x	x	x	x						x	x	x		x	x	x					x		x
South German E-MRSA (131/98)	1	Human	228	5	EP[4]	t001	x	x		x	x	x	x				x			x	x	x					x		x
Rhine Hesse E-MRSA-3 (2387/00)	1	Human	5	5	EP[4]	t002	x	x	x	x			x	x	x					x	x	x						x	
Barnim E-MRSA-15 (1678/96)	1	Human	22	22	EP[4]	t032	x	x		x			x	x						x	x								
Barnim E-MRSA-15 (clinical isolates)	7	Human	22	22	n.t.	n.t.	x	x		x			v	x						x	x		v						
IMT-12048	1	Horse	22	22	BE 2[5]	t032	x	x		x				x						x	x								
IMT-10652	1	Horse	1117	22	IMT-A	t022	x	x		x			x	x						x	x								
IMT-12553	1	Horse	398	398	NT[6]	t011	x			x								x		x	x								

Strain ID	Miscellaneous resistance genes												Superantigenic toxins									Leukocidin/hemolysin gamma locus				Leukocidins		
	sat	dfrA	far1	mupR	tetK	tetM	cat	cfr/fexA	fosB	qacA	qacC	vanA/B/Z	tst	sea	sea-N315(sep)	seb/k/q	sec/l	sed/j/r	see	seg/i/m/n/o/u	seh	lukF	lukS	lukS-ST22+ST45	hlgA	lukF/S-PVL	lukF-PBD/lukM	lukD/E
North German E-MRSA (134/93)						x			x	x				x								x	x	v	x			x
IMT-6634; -8142		x				x			x							x						x	x	v	x			x
IMT-10697		x				x			x							x						x	x	v	x			x
Hannover E-MRSA (1000/93)	x				x	x	x		x							x						x	x	v	x			x
Hannover E-MRSA (clinical isolates)	x					x	v		x					x		x						x	x	v	x			x
IMT-11900; -11901; -8940	x					x			x							x						x	x	v	x			x
IMT-8936	x					x			x							x						x	x	v	x			x
IMT-10029; -8591; -6638; -6637; -9658; -11918	x					x			x							x						x	x	v	x			x
IMT-6636	x					x			x							x						x	x	v	x			x
IMT-12562	x	x				x			x							x						x	x		x			x
IMT-9762	x					x			x							x						x	x	v	x			x
Vienna E-MRSA (635/93)	x					x			x													x	x	v	x			x
South German E-MRSA (131/98)	x								x	x										x		x	x	v	x			x
Rhine Hesse E-MRSA-3 (2387/00)									x						x			x		x		x	x	v	x			x
Barnim E-MRSA-15 (1678/96)																	x			x		v		x	x			
Barnim E-MRSA-15 (clinical isolates)																	v			x				x				
IMT-12048																				x				x	v			
IMT-10652																				x				x				
IMT-12553					x	x																v	x	x	x			

Strain ID	Hemolysins						Miscellaneous virulence factor							Proteases					
	hl	hla	hld	hlIII-all	hlb	un-truncated hlb	sak	chp	scn	etA/B/D	edinA/B/C	arcA/B/C/D	mprF-Col+MW2	mprF-Mu50+252	aur-MRSA252	aur-others	splA/B/E	splE	sspA/B/P
North German E-MRSA (134/93)	x	x	x	x	x		x	x	x				x	v		x	x	x	x
IMT-6634; -8142	x	x	x	x	x	x			v				x	v		x	x	x	x
IMT-10697	x	x	x	x	x	x							x	v		x	x	x	x
Hannover E-MRSA (1000/93)	x	x	x	x	x	x			v				x	v		x	x	x	x
Hannover E-MRSA (clinical isolates)	x	x	x	x	x		x		x				x	v		x	x	x	x
IMT-11900; -11901; -8940	x	x	x	x	x	x							x	v		x	x	x	x
IMT-8936	x	x	x	x	x	x			v				x	v		x	x	x	x
IMT-10029; -8591; -6638; -6637; -9658; -11918	x	x	x	x	x	x			v				x	v		x	x	x	x
IMT-6636	x	x	x	x	x	x			v				x	v		x	x	x	x
IMT-12562	x	x	x	x	x	x							x	v		x	x	x	x
IMT-9762	x	x	x	x	x	x			v				x	v		x	x	x	x
Vienna E-MRSA (635/93)	x	x	x	x	x		x	x	x				x	v	x		x	x	x
South German E-MRSA (131/98)	x	x	x	x	x		x	x	x				v	x		x	x		x
Rhine Hesse E-MRSA-3 (2387/00)	x		x	x	x		x	x	x				v	x		x	x		x
Barnim E-MRSA-15 (1678/96)	x	x	x		x	x		x					v	x	x				x
Barnim E-MRSA-15 (clinical isolates)	x	x	x		x		x	x	x				v	x	x				x
IMT-12048	x	x	x		x		x	x	x					x	x				x
IMT-10652	x	x	x	v	x		x	x	x				v	x	x				x
IMT-12553	x	x	x	x		v							v	x	x				x

Tab. 2: *agr* variants, toxins, superantigens, capsule-, MSCRAMM- and biofilm genes

Strain ID	Number	Host	MLST[1]	Clonal Complex	PFGE-Typ[2]	spa-Typ[3]	Accessory gene regulator locus				Staphylococcal superantigen-like or exotoxin-like-genes																									
							agrI	agrII	agrIII	agrIV	setC	ssl1-COL	ssl1-MU50	ssl1-others	ssl2	ssl2-MRSA252	ssl3	ssl3-MRSA252	ssl4	ssl4-MRSA252	ssl6	ssl7	ssl7-AF188836	ssl8/set12	ssl9	ssl9-MRSA252	ssl10	ssl10-others	ssl11-COL	ssl11-Mu50	ssl11-MRSA252	setB3	setB3-MRSA252	setB2	setB2-MRSA252	setB1
North German E-MRSA (134/93)	1	Human	247	8	EP[4]	t051	x				x	x	v		x	v	x		x		x	x	v	x	x		x	v	x			x		x	x	x
IMT-6634; -8142	2	Horse	8	8	IMT-H/-H-1	t064	x				x	x			x		x		x		x	x	v	x	x		x		x			x		x	x	x
IMT-10697	1	Horse	8	8	IMT-H	t064	x				x	x			x	v	x		x		x	x	v	x	x		x		x			x		x	x	x
Hannover E-MRSA (1000/93)	1	Human	254	8	EP[4]	t009	x				x	x	v		x	v	x		x		x	x	v	x	x		x	v	x			x		x	x	x
Hannover E-MRSA (clinical isolates)	5	Human	254	8	n.t.	n.t.	x				x	v		v	x	v	x		x		x	x	v	x	x		x		x			x		x	x	x
IMT-11900; -11901; -8940	3	Horse	254	8	IMT-G	t009	x				x	x	v		x		x		x		x	x	v	x	x		x	v	x			x		x	x	x
IMT-8936	1	Horse	254	8	IMT-G	t009	x				x	x	v		x		x		x		x	x	v	x	x		x		x			x		x	x	x
IMT-10029; -8591; -6638; -6637; -9658; -11918	6	Horse	254	8	IMT-G/-G-1	t036	x				x	x			x		x		x		x	x	v	x	x		x		x			x		x	x	x
IMT-6636	1	Horse	254	8	IMT-G	t036	x				x	x	v		x	v	x		x		x	x	v	x	x		x		x			x		x	x	x
IMT-12562	1	Horse	254	8	IMT-G	t036	x				x	x	v		x	v	x		x		x	x	v	x	x		x		x			x		x	x	x
IMT-9762	1	Horse	254	8	IMT-G	t036	x				x	x			x		x		x		x	x		x	x		x		x			x		x	x	x
Vienna E-MRSA (635/93)	1	Human	239	8	EP[4]	t037	x				x	x	v	v	x	v	x		x		x	x	v	x	x		x	x	x			x		x	x	x
South German E-MRSA (131/98)	1	Human	228	5	EP[4]	t001		x			x		x	x	x	v	x		x			x	v	x	x		x	v		x		x		x	x	x
Rhine Hesse E-MRSA-3 (2387/00)	1	Human	5	5	EP[4]	t002		x			x	v	x	x	x	v	x		x			v	v	x	x		x	x		x		x		x	x	x
Barnim E-MRSA-15 (1678/96)	1	Human	22	22	EP4	t032	x				x				v	x		x		x	x	v	v		x		v	x								x
Barnim E-MRSA-15 (clinical isolates)	7	Human	22	22	n.t.	n.t.	x				x				x	x				v		v	x		v											
IMT-12048	1	Horse	22	22	BE 25	t032	x				x				x	x		v		x		v	x		x			x								
IMT-10652	1	Horse	1117	22	IMT-A	t022	x				x				x	x					x	v	x		v			x								
IMT-12553	1	Horse	398	398	NT[6]	t011	x				x		x	x		x				x	x	v	x			x		x					x			x

Strain ID	Typ-I site specific DNAse subunit			Capsule genes			Biofilm genes		Genes encoding clumping factor					
	hsdS1-RF122	hsdS2-Sti5+ST8	hsdS-CC15	capH/I/J/K1	capH/I/J/K5	capH/I/J/K8	icaA/C/D	bap	bbp-all	bbp-COL+MW2	clfA-all	clfB-all	clfB-COL+Mu50	clf-MW2
North German E-MRSA (134/93)		x			x		x				x	x	x	
IMT-6634; -8142		x			x		x		x	x	x	x	x	
IMT-10697		x			x		x		x	x	x	x	x	
Hannover E-MRSA (1000/93)		x			x		x				x	x	x	
Hannover E-MRSA (clinical isolates)		x			x		x				x	x	x	
IMT-11900; -11901; -8940		x			x		x				x	x	x	
IMT-8936		x			x		x				x	x	x	
IMT-10029; -8591; -6638; -6637; -9658; -11918		x			x		x				x	x	x	
IMT-6636		x			x		x				x	x	x	
IMT-12562		x			x		x				x	x	x	
IMT-9762		x			x		x					x	x	
Vienna E-MRSA (635/93)		x				x	x		x	x	x	x	x	
South German E-MRSA (131/98)		x			x		x		x	x	x	x	x	
Rhine Hesse E-MRSA-3 (2387/00)		x			x		x		x	x	x	x	x	
Barnim E-MRSA-15 (1678/96)		x			x		x		x	x	x	x	x	
Barnim E-MRSA-15 (clinical isolates)		x			x		x		x	x	x	x	x	
IMT-12048		x			x		x		x	x	x	x	x	
IMT-10652		x			x		x		x	x	x	x	x	
IMT-12553			x		x		x		x	x	x	x	x	x

Strain ID	Collagen-bin.	Coagulase	Endothecell-binding		Genes enconding fibrinogen or fibronectin-binding proteins						extracellular adherence		IgG-binding proteins		Surface-protein-G		Serine aspartate repeat proteins				van Willebrand factor-binding proteins		
	cna	Coagulase, csA	ebh	ebh-RF122	fib	fib-MRSA252	fnbA-COL	fnbA-Mu50+MW2	fnbB-COL	fnbB-others	map-COL	map-others	proteinA	sbi	sasG-COL+Mu50	sasG-MW2	sdrC-all	sdrD-COL-MW2	sdrD-Mu50	sdrD-others	vwb-COL+MW2	vwb-Mu50	vwb-RF122
North German E-MRSA (134/93)	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
IMT-6634; -8142	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
IMT-10697	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
Hannover E-MRSA (1000/93)	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
Hannover E-MRSA (clinical isolates)	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
IMT-11900; -11901; -8940	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
IMT-8936	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
IMT-10029; -8591; -6638; -6637; -9658; -11918	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
IMT-6636	x	x	x	x	x		x		x		x		x	x	x		x	x			x	x	
IMT-12562	x	x	x	x	x		x		x		x		x	x	x		x	v			x	x	
IMT-9762	x	x	x	x	x		x		x		x		x	x	x		x	x					
Vienna E-MRSA (635/93)	x	x	x	x	x		x		x		x		x	x	x		x				x	x	
South German E-MRSA (131/98)	x	x	x	x	x	v			x		x		x	x	x		x		x				
Rhine Hesse E-MRSA-3 (2387/00)	x	x	x	x	x				x		x		x	x	x		x		x				
Barnim E-MRSA-15 (1678/96)	x	x	x	x		x		x		x		x	x	x		x	x			x			x
Barnim E-MRSA-15 (clinical isolates)	x	x				x		x		x		x	x	x		x	x			x			x
IMT-12048	x	x				x		x		x		v	x	x		x	x			x			x
IMT-10652	x	x				x		x		x			x	x		x	x			x			x
IMT-12553	x	x	x	x		x						x	x	x			x		x				

Abbreviations: NT: not typeable; IMT: Institut für Mikrobiologie und Tierseuchen; n.t.: not tested[1]MLST: Multilocus sequence type (ST); [2]Pulsed field gelelectrophoresis, [3]according to ridom *spa* server; [4]EP: Human epidemic strain

Materials and Methods

PFGE, MLST and *spa*- typing

19 strains of equine origin have been collected between 2002 and 2007 from diagnostic specimens. MRSA strains were investigated by PFGE analysis according to Mulvey et al. (6). For all MRSA strains, *spa*-types were determined according to Harmsen and co-workers (2). MLST was performed on representative strains of each different PFGE respectively spa-type as described by Enright and co-workers (1).

Microarray hybridization

The DNA microarray used in this study has been published previously (5). Briefly, it covered probes for 185 distinct genes and 300 alleles, including species-specific controls, accessory gene regulator (*agr*) alleles, genes encoding virulence factors and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), capsule type-specific genes, as well as antimicrobial resistance determinants (AT system by CLONDIAG, Jena, Germany).
More detailed protocols and procedures have been described previously (3, 4).

Results

Analysis of MLST, PFGE, *spa*-typing and microarray hybridisation results of human and equine MRSA revealed that all 19 investigated equine MRSA strains were genetically related with isolates of human origin. Equine strains belonged to clonal complexes (CC) 8 and 22 as well as to ST398.

A large number of similarities in genetic composition were detected in human and equine strains sharing the same genetic background: Lineages ST254/ST8 (CC8) strains of equine origin closely resembled ST254 strains of human origin, especially the Hannover EMRSA strain (Tables 1 and 2: blue letters).

For equine ST254 isolates, SCCmec-typing was renounced due to the lack of detectable recombinase (*ccr*) genes. Both, the absence of ccrA/B2 and the presence of the *mer* operon (Table 1) were also observed in some Hannover EMRSA of human origin. Three strains, belonging to ST8/t064, harbored regular SCC*mec*IV elements.
Two strains belonged to CC22 (ST22 and novel ST1117). Essentially they showed results identical to human MRSA of the Barnim EMRSA type (5). In addition, they carried SCC*mec*IV elements as well as the beta-lactamase operon. (Tables 1 and 2: green letters).

A further strain belonged to ST398 and carried SCC*mec*V (Table 1 and 2).

References

1. **Enright, M. C. et al.** (2000): **J**. Clin. Microbiol. **38:**1008-1015.
2. **Harmsen, D. et al.** (2003): J. Clin. Microbiol. **41:**5442-5428.
3. **Monecke, S. et al.** (2007): Clin. Microbiol. Infect. **13:**236-249.
4. **Monecke, S., and R. Ehricht.** (2005): Clin. Microbiol. Infect. **11:**825-833.
5. **Monecke, S. et al.** (2008): Clin. Microbiol. Infect. **14:**534-545.
6. **Mulvey, M. R. et al.** (2001): J. Clin. Microbiol. **39:**3481-3485.
7. **Sung, J. M. et al.** (2008): Microbiology **154:**1949-1959.
8. **Walther, B. et al.** (2006): Berl. Münch. Tierärztl. Wochenschr. 119:222-232.
9. **Walther, B. et al.** (2008). Vet. Microbiol. **127:**171-178.

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