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Increased genetic diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from companion animals

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ABSTRACT

The aim of the present study was to investigate the diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) that originated from Austrian companion animals during the last five-year period. A total of 90 non-repetitive MRSA isolates were obtained during diagnostic activities from autumn 2013 to autumn 2018. They originated from horses (n = 62), cats (n = 13), dogs (n = 10), rabbits (n = 2), a domestic canary, a zoo-kept hammer-headed bat (*Hypsignathus monstrosus*) and a semi-captive northern bald ibis (*Geronticus eremita*). Antimicrobial susceptibility testing was performed. All isolates were *mecA*-positive and *mecC*-negative. The isolates were genotyped by SCC*mec*, *spa* and *dru* typing, Multiple-Locus Variable number of tandem repeat Analyses (MLVA), *S. aureus* DNA microarray, and whole-genome sequencing (WGS). Eight sequence types (STs - ST398, ST5275 (new ST), ST225, ST8, ST22, ST152, ST1, and ST45), three SCC*mec* types (II, IV, and V), sixteen *spa* types (t003, t008, t011, t015, t032, t034, t1381, t1928, t1985, t223, t334, t355, t430, t6447, t6867, and t7105), fourteen *dru* types (dt10a, dt10az, dt10q, dt10r, dt11a, dt5e, dt6j, dt9a, dt9ak, dt9g, and four new types dt8as, dt7ak, dt4j, dt14n), and thirty-five MLVA types were detected. WGS-based core genome MLST (cgMLST) displayed five main clusters. Compared to the time period 2004–2013, the results of the present study show not only a higher diversity among the MRSA isolates within the population of Austrian companion animals, but also the introduction of new clones. Although ST398 isolates remained predominant, mainly due to high presence of this lineage among horses, increasing isolation rates of human-associated MRSA clones were observed in cats and dogs.

1. Introduction

Methicillin-resistant *Staphylococcus* (*S.*) *aureus* (MRSA) is a major antimicrobial-resistant pathogen responsible for a wide variety of infections worldwide, ranging from mild skin infections to life-threatening diseases. Various studies demonstrated that MRSA clones associated with companion animals, especially dogs and cats, are similar to those identified in humans and that their close companionship increases the risk of co-colonization with *S. aureus* (Loeffler and Lloyd, 2010; Monecke et al., 2011; Walther et al., 2012; Bierowiec et al., 2016). Recent European studies also revealed that among horses, the majority of clinical MRSA isolates belong to the clonal complex (CC) 398, a clade of livestock-associated MRSA (LA-MRSA) (Haenni et al., 2017; Loncaric et al., 2014; Vincze et al., 2014). Equine CC398 from Austria (Loncaric et al., 2014) originated from horses kept as companion animals since the tradition of eating horse meet in Austria is rare. LA-MRSA raised substantial attention during the last decades in human and veterinary medicine. In Austria, the ST398 lineage has been detected in 1.2 % food

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Table 1
Summarized molecular characterization, antimicrobial resistance and toxins profile of the 90 methicillin-resistant *S. aureus* isolates investigated.

Isolates	Host	Source	CC[1]	ST[2]	SCC*mec*	*spa*	*dru*	Antimicrobial resistance profile				
								Phenotype	Genes detected	Amino acid alterations in QRDRs[3]	Biocide resistance genes	Toxins detected
3587	horse	abscess	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
2259	horse	cornea	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
59	horse	mouth	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
1005	horse	tracheal lavage	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
1930	horse	umbillical abscess	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
2700	horse	uterus	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
1840	horse	vein content	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
3915	horse	nose	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
609	horse	nose	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
396, 407, 561, 625, 1225, 1226, 1735, 1874, 1975, 2051, 2201, 2297, 2541, 2834, 2933, 3071, 3232, 3290, 3586, 3877, 1287, 1675	horse	wound	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
3260, 3861, 760	horse	fistula	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
2646	horse	fibrin	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L	*qacC*	
3217	cat	wound	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
2075	domestic canary	eye	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
2200	horse	sinus	CC398	398	IV	t011	dt10q	BLA, GEN, CIP	*mecA, blaZ, blaI, blaR, aacA-aphD*	GrlA S80 F; GyrA S84L		
2517	horse	wound	CC398	5275*	IV	t011	dt10q	BLA, GEN, CIP	*mecA, blaZ, blaI, blaR, aacA-aphD*	GrlA S80 F; GyrA S84L		
10	horse	nose	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
22	horse	synovia	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
290	horse	exudat	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *aacA-aphD***	GrlA S80 F; GyrA S84L		
2202	horse	wound	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *aacA-aphD*, *erm*(C)	GrlA S80 F; GyrA S84L		
3855	horse	wound	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *aacA-aphD*, *erm*(C)	GrlA S80 F; GyrA S84L		
2281	dog	wound	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *aacA-aphD*, *erm*(C)	GrlA S80 F; GyrA S84L		

(*continued on next page*)

Table 1 (*continued*)

Isolates	Host	Source	CC[1]	ST[2]	SCC*mec*	*spa*	*dru*	Antimicrobial resistance profile				
2924	rabbit	eye	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP, SXT	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
3029	horse	sinus	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP, SXT	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
3214	horse	wound	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *aacA-aphD*, *erm*(C)	GrlA S80 F; GyrA S84L		
1281	horse	nose	CC398	398	IV	t011	dt10q	BLA, TET, GEN	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*			
1492	northern bald ibis	feces	CC398	398	IV	t011	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
1418	horse	tracheal lavage	CC398	398	IV	t011	dt10q	BLA, TET, GEN	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*			
3779	horse	wound	CC398	398	IV	t011	dt10q	BLA, TET, GEN	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*			
1728	horse	wound	CC398	398	IV	t011	dt11a	BLA, TET, GEN	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*			
121	rabbit	nose	CC398	398	IV	t011	dt4j**	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
3179	horse	wound	CC398	398	IV	t011	dt7ak**	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
4042	horse	wound	CC398	398	IV	t011	dt8as**	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L	*qacC*	
2656	horse	wound	CC398	398	IV	t011	dt9ak	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
2182	horse	fistel	CC398	398	IV	t011	NT	BLA, TET, GEN				
588	horse	periorbital wound	CC398	398	IV	t1985	dt10q	BLA, TET, GEN, CIP	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*	GrlA S80 F; GyrA S84L		
3058	horse	wound	CC398	398	IV	t6867	dt10q	BLA, TET, GEN	*mecA, blaZ, blaI, blaR, tet*(M), *aacA-aphD*			
38	horse	wound	CC398	398	V	t011	dt14n**	BLA, TET, GEN	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *aacA-aphD*			
1012	cat	nose	CC398	398	V	t034	dt5e	BLA, TET, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *erm*(A), *erm*(C)			
1034	dog	wound	CC398	398	V	t034	dt6j	BLA, TET, CIP, ERY, CLI, SXT	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *erm*(A)	GrlA S80 F; GyrA S84L		
1435	cat	ascites	CC398	398	V	t1928	dt6j	BLA, TET, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *erm*(A), *erm*(C)	GrlA S80 F; GyrA S84L		
1758	horse	wound	CC398	398	V	t011	dt10q	BLA, TET, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *erm*(B)	GrlA S80 F; GyrA S84L		
337	horse	wound	CC398	398	V	t011	dt11a	BLA, TET	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M)			
3655	horse	wound	CC398	398	V	t034	dt11a	BLA, TET	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M)			
3698	horse	joint	CC398	398	V	t011	dt11a	BLA, TET	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M)			
4023	horse	fistel	CC398	398	V	t011	dt11a	BLA, TET, ERY, CLI	*mecA, blaZ, blaI, blaR, tet*(K), *tet*(M), *erm*(B)			
2824	cat	wound	CC1	1	IV	t1381	dt10a	BLA, TET, ERY	*mecA, blaZ, blaI, blaR, tet*(K), *erm*(C), *aphA3*, *sat*			*ent*(H)
1495	hammer-headed bat	lunge	CC152	152	IV	t355	dt10a	BLA, TET, CHL	*mecA, blaZ, blaI, blaR, tet*(K), cat_{pC221}			PVL

(*continued on next page*)

Table 1 (*continued*)

Isolates	Host	Source	CC[1]	ST[2]	SCC*mec*	*spa*	*dru*	Antimicrobial resistance profile			
2624	horse	ostheosyntthesis	CC152	152	IV	t355	dt10a	BLA, TET, CHL	*mecA, blaZ, blaI, blaR, tet*(K), cat_{pC221}		PVL
3235	cat	wound	CC22	22	IV	t032	dt10q	BLA, CIP	*mecA, blaZ, blaI, blaR*	GrlA S80 F; GyrA S84L	*ent*(G), *ent*(I),*ent*(M), *ent*(N), *ent*(O), *ent*(U), *egc*(total)
3278	cat	ear	CC22	22	IV	t7105	dt10a	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(C)	GrlA S80 F; GyrA S84L	*ent*(C), *ent*(G), *ent*(I), *ent*(L), *ent*(M), *ent*(N), *ent*(O), *ent* (U), *egc*(total)
593	cat	skin	CC22	22	IV	t223	NT	BLA	*mecA, blaZ, blaI, blaR, lnu*(A)		*tsst*1, *ent*(G), *ent*(I),*ent*(M), *ent*(N), *ent*(O), *ent*(U), *egc* (total)
3448	cat	subcutane	CC45	45	IV	t015	NT	BLA	*mecA, blaZ, blaI, blaR*		*ent*(C), *ent*(G), *ent*(I), *ent*(L), *ent*(M), *ent*(N), *ent*(O), *ent* (U), *egc*(total)
306	dog	urine	CC5	225	II	t003	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A), *aadD*	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
1846	cat	wound	CC5	225	II	t003	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
1847	dog	urine	CC5	225	II	t003	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
2901	dog	screw post operative	CC5	225	II	t003	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
2984	cat	urine bladder rupture	CC5	225	II	t003	dt10q	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
3268	dog	urine bladder	CC5	225	II	t003	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
3376	dog	urinary calculus	CC5	225	II	t003	dt10r	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
3525	horse	tracheal lavage	CC5	225	II	t003	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
2838	dog	claw fracture	CC5	225	II	t003	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
2756	cat	wound	CC5	225	II	t6447	NT	BLA, CIP, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)	GrlA S80 F; GrlB P587S; GyrA S84L	*ent*(D), *ent*(G), *ent*(I), *ent*(J), *ent*(L), *ent*(M), *ent*(N), *ent* (O), *ent*(R), *ent*(U), *egc*(total)
112	dog	inner ear	CC8	8	IV	t430	dt10az	BLA, TET, GEN, CIP, ERY, CLI, CHL	*mecA*, *tet*(K), *aacA-aphD*, *erm* (C), cat_{pC194}	GrlA S80 F; GyrA S84L	
1038	dog	knee	CC8	8	IV	t008	dt9g	BLA, CIP, ERY	*mecA, blaZ, blaI, blaR, msr*(A), *mph*(C), *aphA3, sat*	GrlA S80Y; GyrA S84L	*ent*(K)

(*continued on next page*)

Table 1 (*continued*)

Isolates	Host	Source	CC[1]	ST[2]	SCC*mec*	*spa*	*dru*	Antimicrobial resistance profile			
1048	cat	ear	CC8	8	IV	t008	dt9g	BLA, CIP, ERY	*mecA, blaZ, blaI, blaR, msr*(A), *mph*(C)	GrlA S80Y; GyrA S84L	PVL, *ent*(K), *ent*(Q)
4454	cat	skin	CC8	8	IV	t334	dt9a	BLA, ERY, CLI	*mecA, blaZ, blaI, blaR, erm*(A)		

BLA = β-lactams; CIP = ciprofloxacin; CHL = chloramphenicol; CLI = clindamycin; ERY = erythromycin; GEN = gentamicin; SXT = trimethoprim-sulfamethoxazole; TET = tetracycline.
[1]clonal complex.
[2]sequence type.
[3]quinolone resistance-determining regions (GrlA, GyrA, GrlB and GyrB); S = Ser, F = Phe, L = Leu, P = Pro, Y = Tyr.
NT = non typeable.
* new sequence type.
** new dru type.
*** obtainded after wgs.

samples and 5–10 % of dust samples in pig breeding facilities (Springer et al., 2009), but information on the presence of MRSA in Austrian animals is still limited. Since the first detection of human clinical LA-MRSA in Austria in 2006 (Springer et al., 2009), LA-MRSA was reported to account for 10.8% of human clinical MRSA isolates in 2013 (Ruppitsch et al., 2017). Kinross et al. (2017) recommended the inclusion of veterinary isolates for LA-MRSA monitoring to document potential reservoirs and transmission pathways. Nevertheless, there is still no nationwide comprehensive monitoring program in Austria that allows the assessment of antimicrobial resistance in companion animals or the evaluation of MRSA rates in food-producing animals. Although an annual official situation report on antimicrobial resistance (AURES) is published by the Austrian Federal Ministry of Health in place (AURES, 2017), there is still lack of information about MRSA in animals. Five years ago, our group presented the first study on MRSA associated with companion animals and horses, which until now is still the only comprehensive study reporting on Austrian clinical MRSA in those animals (Loncaric et al., 2014). Hence, the aim of the present study was to investigate the molecular epidemiology of MRSA, isolated during routine diagnostic bacteriological examinations from various animal samples received at the Institute of Microbiology at the University of Veterinary Medicine in Vienna, over the last five-years.

2. Material and methods

2.1. Isolation of MRSA and antimicrobial susceptibility testing

Between autumn 2013 and autumn 2018, a total of 90 non-repetitive MRSA isolates were recovered from samples originating from companion animals (Table 1) during routine bacteriological diagnostics. All equine samples originated from non-food producing horses. All these clinical samples were received from third parties and, therefore, not subject to reporting obligations of the Ethics and Animal Welfare Commission of the University of Veterinary Medicine in Vienna. All MRSA isolates were stored at −80 °C until further examination. Agar disk diffusion was performed according to the recommendations given in the CLSI document M100 (28th ed.) (Clinical and Laboratory Standards Institute (CLSI), 2018) using the following disks (Beckton Dickinson, Heidelberg, Germany): penicillin (PEN, 10 IU), cefoxitin (FOX, 30 µg), gentamicin (GEN, 10 µg), erythromycin (ERY, 15 µg), clindamycin (CLI, 2 µg), tetracycline (TET, 30 µg), ciprofloxacin (CIP, 5 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), chloramphenicol (CHL, 30 µg), and linezolid (LZD, 30 µg). The reference strain *S. aureus* ATCC®25923 served as a quality control strain.

2.2. Molecular characterization of MRSA

Prior to DNA extraction, isolates were grown on BD Columbia III agar with 5% sheep blood (Beckton Dickinson) and incubated overnight at 37 °C. Bacterial cells were enzymatically lysed and DNA extraction was performed using commercially available spin columns (GenElute™ Mammalian Genomic DNA Miniprep Kits, Sigma-Aldrich, Vienna, Austria) as previously described (Loncaric et al., 2014). Methicillin resistance was confirmed by PCRs with primers targeting *mecA* and *mecC* as described elsewhere (Loncaric et al., 2019). All isolates were genotyped by *spa* typing (Loncaric et al., 2014), *dru* typing (Goering et al., 2008), and Multiple-Locus Variable number of tandem repeat Analyses (MLVA) (Schauer et al., 2018). The presence of φ3 bacteriophage was determined by PCR (Lekkerkerk et al., 2015). A DNA microarray (*S. aureus* Genotyping Kit 2.0, Alere, Jena, Germany) was used to identify more than 330 species-specific, virulence-associated, and resistance genes (Monecke et al., 2008). For visualization the diversity between the DNA microarray results (Coombs et al., 2010) the program SplitsTree4 (Huson and Bryant, 2006) was used. For comparative genomics, the Seqsphere + software (Ridom, Münster, Germany) was

used for whole-genome sequence (WGS)-based core genome multilocus sequence typing (cgMLST) and calculation of minimum spanning trees (MST) as previously described (Leopold et al., 2014; Lepuschitz et al., 2017, 2018). Information on the classical MLST was also extracted from WGS data. The presence of antimicrobial resistance and virulence genes was extracted from WGS data via comparison with the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017) or based on Alere Microarray data (Strauß et al., 2016).

3. Results

3.1. MRSA isolates and antimicrobial susceptibility testing

Most MRSA isolates originated from horses (n = 62, 68.9%). A total of 13 (14.4%) MRSA isolates were recovered from cats while ten (11.1%) MRSA isolates originated from dogs. The remaining MRSA isolates were from rabbits (n = 2, 2.2%), a domestic canary (1.1%), a zoo-kept hammer-headed bat (*Hypsignathus monstrosus*) (1.1%) and a semi-captive northern bald ibis (*Geronticus eremita*) (1.1%).

Overall, all 90 MRSA isolates were susceptible to linezolid and all but three were susceptible to chloramphenicol. Most of the isolates were resistant to ciprofloxacin (n = 72, 80.0%), and tetracycline (n = 71, 78.9%) (Table 1). The most common resistance phenotype included resistance to β-lactams together with ciprofloxacin, gentamicin and tetracycline (Table 1). The majority of isolates displayed a multi-drug resistance phenotype (n = 84, 93.3%) (Sweeney et al., 2018).

The detection of resistance genes reflected well the phenotypic resistance profiles of the respective isolates. Besides *mecA*, which was present in all MRSA isolates, the resistance genes *blaZ*, *tet*(M) and *aacA-aphD* were frequently detected (Table 1). In all ciprofloxacin-resistant isolates, mutations in the quinolone resistance-determining regions (QRDRs) of the genes *gyrA*, *grlA* and *grlB* were observed (Table 1) To the best of our knowledge, some of the resistance genes were detected for the first time among MRSA isolates originating from companion animals and horses, namely *lnu*(A), *msr*(A), *mph*(C), *aadD*, *aphA3*, *sat*, cat_{pC194}, and cat_{pC221} (Table 1). The WGS detected additional resistance genes including: *dfrK*, *dfrG*, *spc*, *lnu*(B), *lsa*(E), *str* and *sat4* (Table S2). Antiseptic resistance profiling revealed that all 90 isolates lacked *qacA*, while two isolates carried *qacC*.

3.2. Molecular characterization of MRSA

All MRSA isolates investigated were *mecA*-positive and *mecC*-negative. Clonal complex (CC) 398 was the most dominant CC identified (n = 69, 76.7%), followed by a broad diversity of nine other CC's among the remaining 21 isolates.

CC398 isolates were of equine origin and based on WGS belonged either to ST398 (n = 59) or to the new ST5275 (n = 1), feline (ST398, n = 4), canine (n = 2), and leporine origin (n = 2). The remaining ST398 isolate was from a northern bald ibis. The majority of the ST398 isolates (n = 59), and the single ST5275 isolate harboured SSC*mec* type IV elements whereas the remaining nine ST398 isolates carried SSC*mec* type V elements. In total, 62 of the 68 ST398 isolates as well as the ST5275 isolate were identified as *spa* type t011, whereas the other six ST398 isolates belonged to t034 (n = 3), t1928, t1985, and t6867. Fifty-four of the ST398 isolates and the ST5275 isolate belonged to *dru* type (dt) 10q. Other ST398 isolates carried dt11a (n = 5) or dt6j (n = 2), while single ST398 isolates were assigned to dt5e, dt9ak or any of the four new *dru* types dt8as, dt7ak, dt4j, and dt14n. In one ST398 isolate, the *dru* region could not be amplified by PCR.

The second most common CC was CC5. All isolates within CC5 belonged to ST225 (11.1%) and were of canine (n = 6), feline (n = 3) and equine (n = 1) origin. All ten ST225 isolates carried SCC*mec* II and belonged to either *spa* type t003 (n = 9) or t6447 (n = 1). Interestingly, only in two isolates the *dru* types could be determined (dt10r and dt10q).

The two canine and the two feline CC8 MRSA (4.4%) isolates belonged to ST8, carried SCC*mec* IV, belonged to *spa* type t008 (n = 2), t334 (n = 1), or t430 (n = 1), and carried the *dru* type dt9a (n = 1), dt9g (n = 2), or dt10az (n = 1). Three isolates belonged to CC22 (3.3%) and ST22 and were of feline origin. They carried SCC*mec* IV elements and belonged to three *spa* types (t032, t223, t7105) as well as to two *dru* types (dt10q, dt10a). From one isolate, the *dru* region could not be amplified. The two CC152-ST152 (2.2%) isolates originated from a horse and the zoo-kept hammer-headed bat. They had all characteristics in common, i.e. SCC*mec* IV, *spa* type t355 and *dru* type dt10a. Finally, two feline isolates belonged to either CC1-ST1-SCC*mec* IV-*spa* type t1381-*dru* type dt10a (1.1%) or to CC45-ST45-IV-t015-*dt*NT (*dru* non-typeable) (1.1%), respectively.

MLVA differentiated the examined isolates in 35 types (Table S1). The Immune Evasion Cluster (IEC) (as a marker of human adaptation) was detected in 19 isolates, including type E (n = 9; two of CC152-ST152, and seven of CC5-ST5), type B (n = 6; three of CC22-ST22 and one CC5-ST5, CC398-ST398 and CC45-ST45 isolate), type F (n = 2, two CC5-ST5 isolates), and type G (one isolate of CC8-ST8) (Table S1). In all 19 isolates, the presence of the φ3 bacteriophage was confirmed by PCR

DNA-microarray analyses as well as WGS revealed the presence of several virulence genes. The Panton-Valentine leukocidin (PVL) genes were detected in three isolates, a feline CC8-ST8-IV-t008-dt9g, and in two CC152-ST152-IV-t355-dt10 that originated from the hammer-headed bat and a horse. One feline CC22-ST22-IV-t223-dtNT harboured the toxic shock syndrome toxin 1 gene *tst1*. Fourteen isolates belonging to CC5 (n = 10), CC22 (n = 3) and CC45 (n = 1) carried the enterotoxin gene cluster *egc* comprising the enterotoxin genes *seg*, *sei*, *sem*, *sen*, *seo*, and *seu*. Other enterotoxin genes, including *sea* (N315)/*sep*, *sec*, *sed*, *seh*, *sej*, *sek*, *sel*, *seq*, and *ser* were detected in various isolates (Table 1). None of CC398 isolates harboured any genes coding for staphylococcal enterotoxins.

Splitstree analysis of the microarray data confirmed the strong ST398 presence and showed clonal clustering into seven groups based on their virulence and antimicrobial resistance profile similarities (Fig. S1).

Whole genome-based cgMLST phylogenetic analysis including all isolates was conducted and a minimum spanning tree was calculated (Fig. 1). Distance calculation between all isolates revealed an allelic distance between isolates from zero to a maximum of 1578. Based on the defined cluster threshold (CT) of 24 allelic differences, five different clusters were obtained. Four clusters were generated within CC398, and one within CC5 (Fig. 1).

4. Discussion

The present study describes the characterization of MRSA predominantly isolated from Austrian companion animals such as dogs, cats, and horses, during the last five years. All examined isolates were *mecA*-positive and *mecC*-negative. So far, *mecC*-positive MRSA have only been observed among wild and food-producing animals in Austria (Loncaric et al., 2013a,2013b; Schauer et al., 2018) as well as in humans (Kerschner et al., 2014). Thus, companion animals are still the only animal population in which *mecC*-positive MRSA has not been detected yet.

Results of the present study may be compared with those obtained from a MRSA collection isolated from companion animals between 2004 and 2013 in Austria (Loncaric et al., 2014). The annual average numbers of examined samples originating from companion animals and horses remained constant and thus, enabled comparison. Even though horses are still the most predominant companion animals from which MRSA could be isolated (68.9%), the overall isolation rate of MRSA from horses was distinctly lower than in the previous study period (87.6%). Between 2004 and 2013, the MRSA isolation rate from feline and canine hosts was rarely above 10% but increased between 2013

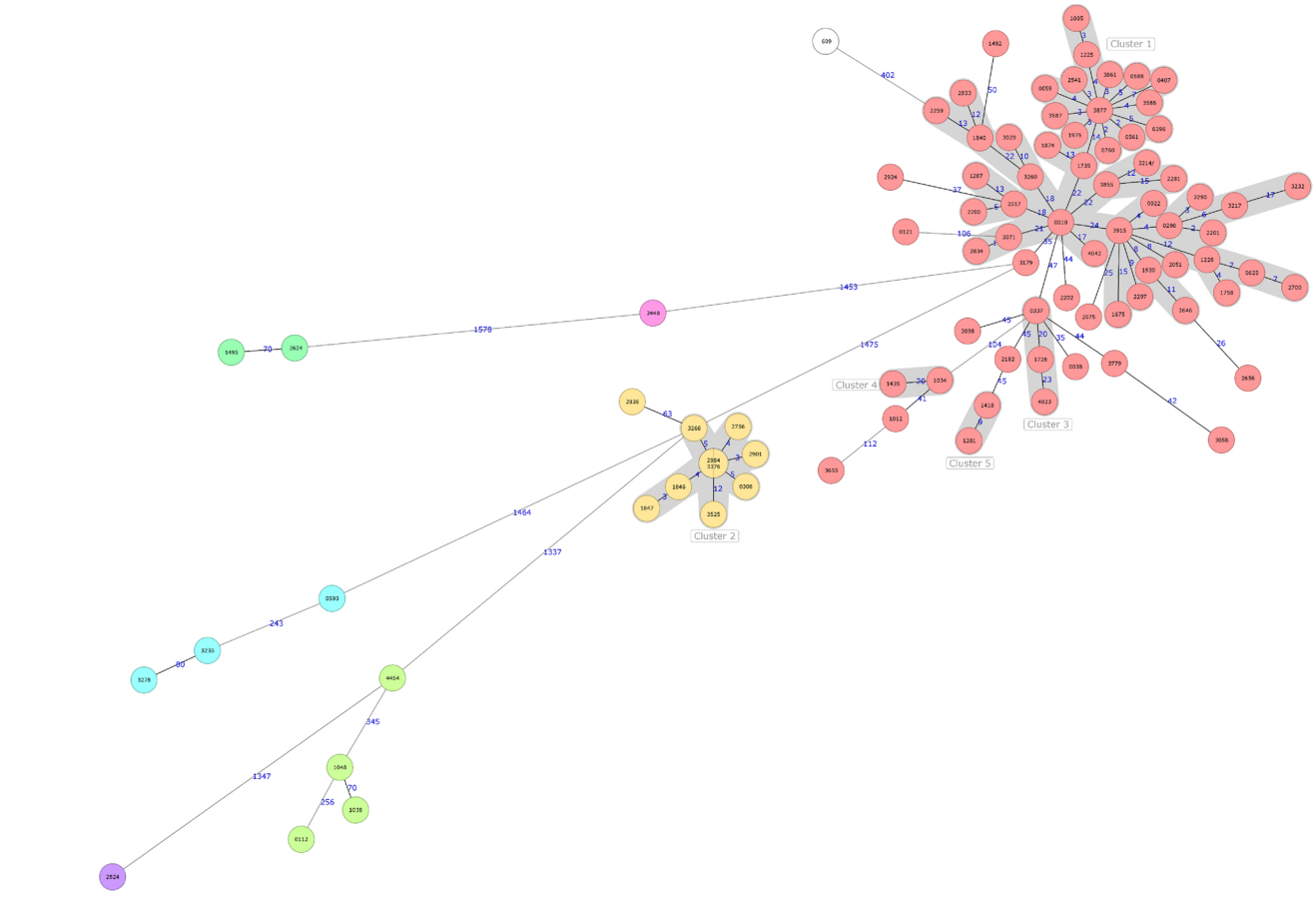

Fig. 1. Minimum spanning tree for 90 MRSA isolates based on the cgMLST of *S. aureus*. Colours correspond to the clonal complex. Each circle represents isolates with an allelic profile based on the sequence of 1,861 core genome targets. Blue numbers refer to the allelic differences between two isolates. Isolates with closely related genotypes were identified with a maximum of 24 allelic differences and are shaded in grey (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

and 2018 to more than 25%. MRSA isolated from other companion animals remained a sporadic observation. In addition, MRSA isolates examined during the recent period were more diverse. Seven STs and sixteen *spa* types were detected during the present study, compared to five STs and nine *spa* types demonstrated in our past study. We also observed the introduction of new MRSA clones in the population of examined animals, belonging to STs 225, 8, 152, 45, during the recent study period. Interestingly, ST254, once the second most prevalent clone among horses in Austria, was not detected during the recent observation period.

The most prevalent MRSA clonal complex was CC398 (76.7%). This high presence of CC398 is mainly due to equine origin. Equine MRSA isolates belonging to other lineages is still rare observation. CC398-IV-t011 remained the major clone among Austrian non-food producing animals. This clone was also shown to be the most predominant MRSA infecting horses in France (Haenni et al., 2017), Germany (Vincze et al., 2014), and Denmark (Islam et al., 2017), replacing CC8. In the current study, the presence of IEC carried by φ3 bacteriophage and related to human origin/adaptation (Jung et al., 2017) was only observed in one equine CC398-V-t011 isolate. This rate (1.4%) is notably low compared to IEC-carrying equine CC398 isolates in France (17.2%) (Haenni et al., 2017) and Germany (~10%) (Cuny et al., 2015). Non-equine CC398 isolates remained a sporadic observation, which correlates with a recent report from France (Haenni et al., 2017).

The second most prevalent MRSA in our study belonged to CC5, a common and widespread clonal complex comprising a variety of different MRSA isolates (Monecke et al., 2011). CC5-ST225-II, detected among MRSA isolates from dogs, cats and a horse in the current study, represents a MLST single locus variant (SLV) of the pandemic ST5 (Monecke et al., 2011). A CC5-MRSA has already been isolated from a cat in the past MRSA study of our working group (Loncaric et al., 2014). Even though isolation rates of CC5 are still low, CC5-MRSA isolates have obviously been established in Austrian companion animals. This clonal complex has also been detected in cats and dogs during similar studies conducted in France (Haenni et al., 2017), Switzerland (Wipf and Perreten, 2016), and Germany (Vincze et al., 2014). In contrast, CC5-MRSA have only been occasionally detected in horses. Due to the presence of IEC, the enterotoxin gene cluster (*egc*), and the high proximity between cats or dogs and their owners, this human-related clone deserves special attention in the years to come.

Another human-related lineage, pandemic CC8 (Monecke et al., 2011), was detected in the present study representing a new introduction into the Austrian companion animal population. While three CC8-ST8-IV isolates were shown to be PVL-negative, one feline CC8-ST8-IV-t008-dt9g was PVL-positive which is widely known as USA300 clone (Monecke et al., 2011). So far, PVL-positive MRSA have only been detected among wildlife in Austria (Loncaric et al., 2013a,2013b). In contrast to studies from France and Germany (Haenni et al., 2017; Vincze et al., 2014), this clone has never been observed in Austrian horses before.

Although considered to be pandemic, CC22-MRSA is still not common in companion animals from Austria, but well established in the human population (Zarfel et al., 2016). In the present study, three feline CC22-ST22-IV were detected, all of them belonging to different *spa* types and carrying different *dru* types and did not cluster together, neither by using MLVA nor after cgMLST. A novel observation was the

presence of the toxic shock syndrome toxin (*tst1*) gene in one feline isolate, which has never been detected before in MRSA originating from Austrian companion animals and horses. Up to now, the *tst1* gene has only been described in an ovine CC45 isolate from Austria (Schauer et al., 2018). The presence of IEC type B suggests that humans might be a likely infection source for cats. The presence of the same IEC in CC22-IV was observed in feline and canine MRSA in France (Haenni et al., 2017).

PVL genes were detected in two isolates of CC152-IV-t355-dt10a, a clone that has never been detected in Austria in the past. CC152 has been sporadically reported in humans and all of these MRSA isolates carried PVL genes but were associated with SCC*mec* V (Monecke et al., 2011). Two CC152-IV-t355-dt10a isolated from a horse and a hammer-headed bat differed in their MLVA profile and did not cluster by cgMLST. Interestingly, the zoo-kept hammer-headed bat was free-flying in a kind of aviary that is highly frequented by zoo visitors. Thus, a human to animal transmission or vice versa could have been occurred.

Two feline singletons belonging to CC1-ST1-IV-t1381-dt10a (isolate 2824) and CC45-ST45-IV-t015-dtNT (isolate 3448) have also been detected during the present study. CC1 has also only rarely been observed in similar studies conducted in France and Germany (Haenni et al., 2017; Vincze et al., 2014). CC1 is known to carry PVL genes (Monecke et al., 2011), but our isolates did not carry those genes. CC45 is one of the major lineages from which several MRSA strains have emerged (Monecke et al., 2011). So far, CC45 has never been detected among companion animals in Austria and has not be observed in a similar study from Germany (Vincze et al., 2014) or was also a singleton in France (Haenni et al., 2017). The presence of IEC in our CC45-ST45-IV-t015-dtNT isolate suggests human origin although this clone is also scarce in the human population of Austria (Zarfel et al., 2016).

5. Conclusion

The present study is the continuation of our past study and is the only and the most comprehensive study dealing with MRSA from companion animals and horses in Austria. In conclusion, MRSA isolated during the last 5 years showed a higher diversity than MRSA isolates examined in the past. MRSA belonging to STs 225, 8, 152, 45 have been introduced into the Austrian companion animal population including horses during the recent study period. Whether these isolates will remain in these animals should be monitored. Our study demonstrated that the majority of isolates recovered from small companion animals and all non−CC398 of equine origin are human-related, mirroring the human MRSA epidemiology. These strains harboured enterotoxins more frequently and displayed a higher presence of the IEC cluster than most of the equine isolates, which belonged rather to animal-associated LA-MRSA. Considering the high proximity between companion animals and humans our results undoubtedly identified a public health issue because once companion animals become affected by MRSA, it can be easily transmitted in both direction, anthroponotic and zoonotic. This deserves to be monitored also in the years to come.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.06.013.

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