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Phenotypic and molecular characterization of *Staphylococcus aureus* strains of veterinary, dairy and human origin

M. GONANO[1], I. HEIN[1]*, P. ZANGERL[2], A. RAMMELMAYR[3] AND M. WAGNER[1]

[1] *Institute of Milk Hygiene, Milk Technology and Food Science, Department of Veterinary Public Health and Food Science, University of Veterinary Medicine, Vienna, Austria*
[2] *Federal Institute for Alpine Dairying, Rotholz, Austria*
[3] *Austrian Agency for Health and Food Safety (AGES), Vienna, Austria*

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SUMMARY

Austrian veterinary ($n=91$), dairy ($n=86$), and human strains ($n=48$) of *Staphylococcus aureus* were tested for various phenotypic properties including clumping factor, egg-yolk reaction, production of thermonuclease and susceptibility to 14 antibiotics. In addition the expression of enterotoxins (A–E), and the presence of enterotoxin genes *sea* to *sej* and *tst* was determined. Significant differences in antimicrobial susceptibility were found with 84·6% of veterinary, 57·0% of dairy, and 20·8% of human strains susceptible to all antibiotics tested ($P<0·0005$). More human strains produced enterotoxins (41·7%) than veterinary (9·9%) and dairy strains (12·6%) while 40·7% and 38·5% of veterinary, 47·7% and 52·3% of dairy, and 77·1% and 87·5% of human strains were *se*- and *tst*-positive, respectively. AFLP analysis revealed nine clusters with over- or under-representation of strains with specific characteristics. Strains clustered according to origin (veterinary, dairy, and human) and/or presence of toxin genes and antimicrobial resistance.

Key words: AFLP, antibiotic resistance, genotyping, *Staphylococcus aureus*, toxins.

INTRODUCTION

Staphylococcus aureus is a leading cause of various purulent infections in humans and animals and is one of the most significant pathogens causing intramammary infections in dairy cattle worldwide. Some strains are able to produce enterotoxins (SE), which belong to the family of staphylococcal and streptococcal pyrotoxins [1]. These toxins can cause food poisoning, toxic shock and also allergic and autoimmune reactions [1–3]. The classical enterotoxins SEA-SEE have been well known as agents of food poisoning for several years. The emetic potential of *S. aureus* expressing the enterotoxin genes *seg* to *sej* is also evident, but their relevance in food intoxication remains unclear [1]. To date, only SEH has been linked to an outbreak due to consumption of Brazilian cheese [4]. In recent years a further 11 genes have been identified: *sek*, *sel*, *sem, sen*, *seo*, *sep*, *seq*, *ser*, *seu*, *seu2* and *sev* [5, 6]. These toxins lack the typical emetic effect although they appear to be enterotoxin-like superantigens. The toxic-shock syndrome toxin (TSST-1), a distantly related protein and

* Author for correspondence: Dr I. Hein, Institute of Milk Hygiene, Milk Technology and Food Science, Department of Veterinary Public Health and Food Science, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria. (Email: ingeborg.hein@vu-wien.ac.at)

the first toxin found to be involved in the toxic-shock syndrome of humans and animals, was also shown to be non-emetic [3].

The monitoring of strains in human and animal reservoirs provides important epidemiological information and several molecular methods have enabled the analysis of marker genes and facilitated the tracing of strains from various sites. Genotyping methods such as ribotyping, restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), and multilocus sequence typing (MLST) have been all been applied to phylogenetic and epidemiological studies [6] and are able to discriminate, to a greater or lesser degree, between unrelated strains [7].

Data from Austria regarding toxin production and antibiotic resistance of human and non-human *S. aureus* strains have been published separately in previous studies but the results of these investigations cannot be compared directly as different methods were used and they focused on different traits of *S. aureus* [8–10]. We set out to characterize Austrian *S. aureus* strains collected from veterinary, dairy and human origin in parallel, with respect to important infection-related traits such as presence of toxin genes, toxin expression, and antimicrobial resistance. Our aim was to gain comparable information about the ecological diversity of staphylococcal populations circulating in human and non-human reservoirs.

MATERIALS AND METHODS

S. aureus strains

Ninety-one veterinary strains, 247 dairy strains, and 48 human strains were included in the study. The veterinary strains originated from cases of bovine mastitis in 2001 and 2002 in Upper Austria that were investigated by the Agency for Health and Food Safety (AGES), Vienna. The dairy strains were isolated from cheese-making milk and raw milk cheeses in the period 1991–1998 by the Federal Institute for Alpine Dairying, Rotholz, and the human strains were from wound infections in 2002 at the Vienna General Hospital. All strains were from separate clinical cases or products.

The presence of clumping factor, egg-yolk reaction, and production of thermonuclease and staphylococcal enterotoxins (SE) A–E was determined for all strains but subsets of 91 veterinary, 86 dairy, and 48 human strains were tested for antimicrobial susceptibility and the presence of *se* genes and *tst* by polymerase chain reaction (PCR) analysis. These strains were also subjected to genotyping by AFLP.

Clumping factor, egg-yolk reaction, and thermonuclease

The presence of clumping factor was determined using the staphylase test (Oxoid, Basingstoke, UK). The egg-yolk reaction was tested for on Baird–Parker agar plates (Oxoid) which were incubated at 37 °C and inspected after 48 h. The production of thermonuclease was detected according to OENORM-DIN 10197 on Toluidine Blue O agar [11].

Antimicrobial susceptibility testing

Susceptibility testing was performed using the agar disc diffusion method on Mueller–Hinton agar (Oxoid) as recommended by the CLSI [12, 13]. For an easier visual interpretation the DIN 58940 protocol was used for oxacillin testing [14]. The following antimicrobial agents and doses were used: amikacin (30 μg), amoxicillin/clavulanic acid (20/10 μg), cefoperazone (30 μg), clindamycin (2 μg), erythromycin (15 μg), enrofloxacin (5 μg), fusidic acid (10 μg), gentamicin (10 μg), ofloxacin (5 μg), oxacillin (5 μg), penicillin G (10 U), vancomycin (30 μg), all obtained from Oxoid, cefquinom (10 μg) from Intervet (Boxmeer, The Netherlands), and marbofloxacin (5 μg) from Vétoquinol (Lure, France). Antimicrobial zone diameters were interpreted according to CLSI, except for cefoperazone, cefquinom, and enrofloxacin in which cases the interpretation criteria of Peracef® (Pfizer, Zürich, Switzerland), Cobactan® (Intervet, Unterschleißheim, Germany) and Baytril® (Bayer, Leverkusen, Germany) were used, respectively.

Immunological detection of enterotoxin production

All strains were tested for the production of enterotoxins A, B, C, D, and E in brain heart infusion broth (BHI; Merck, Darmstadt, Germany) using the Staphylococcal enterotoxin visual immunoassay (VIA™; Tecra International Pty Ltd, French Forest, Australia).

DNA isolation

Genomic DNA was isolated from 1-ml volumes of an overnight culture at 37 °C in BHI using the Qiagen®

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Table 1. *Sequences and modifications of oligonucleotides used for PCR and AFLP*

Analysis	Gene	Oligonucleotide	Sequence and modifications
PCR 1 and PCR 2	*sea*	GSEAR-1	5′-GGT TAT CAA TGT GCG GGT GG-3′
		GSEAR-2	5′-CGG CAC TTT TTT CTC TTC GG-3′
	seb	GSEBR-1	5′-GTA TGG TGG TGT AAC TGA GC-3′
		GSEBR-2	5′-CCA AAT AGT GAC GAG TTA GG-3′
	sec	GSECR-1	5′-AGA TGA AGT AGT TGA TGT GTA TGG-3′
		GSECR-2	5′-CAC ACT TTT AGA ATC AAC CG-3′
	sed	GSEDR-1	5′-CCA ATA ATA GGA GAA AAT AAA AG-3′
		GSEDR-2	5′-ATT GGT ATT TTT TTT CGT TC-3′
	see	GSEER-1	5′-AGG TTT TTT CAC AGG TCA TCC-3′
		GSEER-2	5′-CTT TTT TTT CTT CGG TCA ATC-3′
	seg	SEG1	5′-TGC TAT CGA CAC ACT ACA ACC-3′
		SEG2	5′-CCA GAT TCA AAT GCA GAA CC-3′
	seh	SEH1	5′-CGA AAG CAG AAG ATT TAC ACG-3′
		SEH2	5′-GAC CTT TAC TTA TTT CGC TGT C-3
	sei	SEI1	5′-GAC AAC AAA ACT GTC GAA ACT G-3′
		SEI2	5′-CCA TAT TCT TTG CCT TTA CCA G-3′
	sej	SEJF	5′-CAT CAG AAC TGT TGT TCC GCT AG-3′
		SEJR	5′-CTG AAT TTT ACC ATC AAA GGT AC-3′
PCR 3	*tst*	GTSSTR-1	5′-ACC CCT GTT CCC TTA TCA TC-3′
		GTSSTR-2	5′-TTT TCA GTA TTT GTA ACG CC-3′
AFLP			
Adaptor		*Eco*RI-specific adaptor	5′-CTC GTA GAC TGC GTA CC-NH_2-3′
			5′-P-AAT TGG TAC GCA GTC TAC-3′
		*Mse*I-specific adaptor	5′-GAC GAT GAG TCC TGA G-3′
			5′-TAC TCA GGA CTC AT-NH_2-3′
Pre-selective PCR		*Eco*RI + 0	5′-GAC TGC GTA CCA ATT C-3′
		*Mse*I + 0	5′-GAT GAG TCC TGA GTA A-3′
Selective PCR		*Eco*RI + 0FAM	5′-FAM-GAC TGC GTA CCA ATT C-3′
		*Mse*I + C	5′-GAT GAG TCC TGA GTA AC-3′

AFLP, Amplified fragment length polymorphism; PCR, polymerase chain reaction.

Genomic DNA kit (Qiagen Inc., Valencia, CA, USA) in accordance with the manufacturer's instructions and eluted with ddH_2O. The DNA concentration was determined in a Hoefer DyNA Quant200 apparatus (Pharmacia Biotech, San Francisco, CA, USA) and adjusted with ddH_2O to 1 ng/μl (PCR) or 5 ng/μl (AFLP).

Detection of enterotoxin genes and *tst* by PCR

Strains were tested for the presence of *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei, sej*, and *tst* by PCR as published previously, with modifications [15–17]. Primers were obtained from MWG Biotech (Ebersberg, Germany). Primers were combined to obtain two different sets of multiplex PCRs: one set contained primers for *sea*, *seb*, *sec*, *sed*, and *see* (PCR 1) and the other one for *seg*, *seh*, *sei*, and *sej* (PCR 2). PCR for the detection of *tst* was performed separately (PCR 3). Primers and PCR conditions are shown in Tables 1 and 2. PCR amplification was carried out in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Amplicons were visualized in UV light after electrophoresis in 1·5% agarose gels.

AFLP subtyping of *S. aureus* isolates

A total of 25 ng DNA was digested and ligated for 2 h at 37 °C in a volume of 11 μl containing 1 μl enzyme master mix [50 mM NaCl, 50 mM Tris–HCl (pH 7·8), 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 25 μg/ml BSA, 1 U *Mse*I, 5 U *Eco*RI, and 1 U T4 DNA ligase (New England Biolabs, Beverly, MA, USA)], 1 μl 10 × T4 DNA ligase buffer [500 mM Tris–HCl (pH 7·8), 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP, 250 μg/ml BSA], 1 μl 0·5 M NaCl, 0·5 μl BSA solution (1 mg/ml), 1 μl *Eco*RI restriction site-specific adaptor (2 μM) and 1 μl *Mse*I restriction site-specific adaptor

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Table 2. *PCR conditions used for the amplification of* tst *and see genes and for pre-selective and selective PCR (AFLP analysis)*

PCR analysis	Reaction conditions	Reaction volume	Template DNA	Amplification profile	
PCR 1 and PCR 2	10 mM Tris–HCl	25 μl	2 μl	10 min/95 °C	
	50 mM KCl			30 cycles each	1 min/94 °C
	5 mM $MgCl_2$				1 min/55 °C
	0·001 % (w/v) gelatin				1 min/72 °C
	400 nM of each primer			10 min/72 °C	
	200 μM (each) of dATP, dTTP, dGTP, and dCTP				
	1 U of AmpliTaq Gold™ polymerase*				
PCR 3	10 mM Tris–HCl	25 μl	5 μl	2 min/94 °C	
	50 mM KCl			35 cycles each	1 min/94 °C
	1·5 mM $MgCl_2$				1 min/57 °C
	0·001 % (w/v) gelatin				1 min/72 °C
	400 nM of each primer			7 min/72 °C	
	200 μM (each) of dATP, dTTP, dGTP, and dCTP				
	1·5 U Platinum® *Taq* DNA polymerase†				
AFLP					
Pre-selective	10 mM Tris–HCl	20 μl	4 μl	10 min/94 °C	
	50 mM KCl			20 cycles each	20 s/94 °C
	2·5 mM $MgCl_2$				30 s/56 °C
	0·001 % (w/v) gelatin				2 min/72 °C
	250 nM adaptor-specific primer *Eco*RI + 0				
	2·5 μM adaptor-specific primer *Mse*I + 0				
	1·25 U of AmpliTaq Gold™ polymerase				
Selective	10 mM Tris–HCl	20 μl	3 μl‡		
	50 mM KCl			10 min/94 °C	
	2·5 mM $MgCl_2$			11 cycles each	20 s/94 °C
	0·001 % (w/v) gelatin			touchdown PCR	30 s/66 °C§
	250 nM adaptor-specific primer *Eco*RI + 0FAM				2 min/72 °C
	2·5 μM adaptor-specific primer *Mse*I + C			19 cycles each	20 s/94 °C
	1·25 U of AmpliTaq Gold™ polymerase				30 s/56 °C
					2 min/72 °C
				30 min/60 °C	

AFLP, Amplified fragment length polymorphism; PCR, polymerase chain reaction.
* Applied Biosystems, Foster City, CA, USA.
† Invitrogen, Lofer, Austria.
‡ Amplicons from pre-selective PCR diluted 1:20 with 0·1 × TE buffer.
§ Decreasing 1 °C every cycle until 56 °C was achieved.

(20 μM), 0·5 μl ddH_2O, and 5 μl DNA solution (5 ng/μl). The adaptors were modified to allow exponential amplification of heterosite fragments only [18]. Then 189 μl 0·1 × TE buffer was added to the restriction ligation mixture.

Primers used for pre-selective and selective PCR and PCR conditions are listed in Table 1. Pre-selective and selective PCR was performed in a GeneAmp PCR System 9700 (Applied Biosystems) with the ramp rate set to 90 %.

Amplified DNA fragments were separated by capillary electrophoresis on an ABI-310 Genetic Analyser (Applied Biosystems) with POP4 polymer and GeneScan™ 500 ROX size standard (Applied Biosystems) as the internal standard for each sample. Cluster analysis was performed with the Fingerprinting II software (Bio-Rad, Hercules, CA, USA) using the following settings: band classes from 120 to 400 bp, 0·5% optimization, 0·3% position tolerance, Dice similarity coefficient. For dendrogram construction

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the UPGMA algorithm was used. In addition, a principal components analysis (PCA) based on the binary data of the AFLP analysis was performed using the XLSTAT program (Addinsoft, New York, NY, USA).

Statistical analysis

Differences in the representation of strains with distinctive traits from different origins and within different AFLP clusters were calculated using Fisher's exact test or χ^2 test, as appropriate (SPSS 13.0 statistical software package; SPSS Inc., Chicago, IL, USA). Heterogeneity of the AFLP profiles of different origins was calculated as published previously using Simpson's index of diversity [19].

RESULTS

Clumping factor, egg-yolk reaction, and thermonuclease production

All veterinary strains, 92·3% dairy strains, and 97·9% human strains were clumping factor-positive. Regarding the egg-yolk reaction, 53·8% veterinary strains, 45·3% dairy strains, and 85·4% human strains were positive (veterinary/human and dairy/human, $P<0·0005$; veterinary/dairy, $P=0·165$). As expected, almost all strains produced thermonuclease and only two veterinary and one human strain were negative.

Antimicrobial susceptibility testing

All 225 *S. aureus* strains tested were susceptible to amikacin, amoxicillin/clavulanic acid, cefoperazone, cefquinome, oxacillin, and vancomycin. A total of 136 (60·4%) of the strains were susceptible to all antimicrobial agents tested, including 84·6% of veterinary, 57·0% of dairy, and 20·8% of human strains. These differences were significant ($P<0·0005$). Eleven (12·1%) of the veterinary strains tested were resistant to penicillin G and two strains (2·2%) to fusidic acid. One strain was resistant to both of these antibiotics tested. Penicillin resistance was highest among dairy strains with 35 (40·6%) expressing resistance to this agent; one strain was resistant to erythromycin and another was resistant to penicillin G and fusidic acid. Resistance was most prevalent in human strains; 32 (66·7%) were resistant to penicillin G, one strain was resistant to erythromycin and four (8·3%) strains to both erythromycin and penicillin G. Only one human strain was resistant to more than two different antibiotics: clindamycin, enrofloxacin, erythromycin, gentamicin, marbofloxacin, ofloxacin, and penicillin G.

Table 3. *SE* (*enterotoxin*) *phenotypes of veterinary, dairy, and human* S. aureus *strains*

SE phenotype	No. (%) of strains			
	Mastits	Dairy	Human	Total
SE-negative	82 (90·1)	216 (87·43)	28 (58·3)	326 (84·5)
SE-positive	9 (9·9)	31 (12·6)	20 (41·7)	60 (15·5)
A		6 (2·4)	4 (8·3)	10 (2·6)
B	2 (2·2)	6 (2·4)	6 (12·5)	14 (5·7)
C	6 (6·6)	13 (5·3)	9 (18·6)	28 (7·3)
D	1 (1·1)	1 (0·4)	1 (2·1)	3 (0·8)
AD		4 (1·6)		4 (1·0)
ABC		1 (0·4)		1 (0·3)

Production of enterotoxin A, B, C, D, E, and presence of *se* genes and *tst*

ELISA-based testing revealed that of 386 strains tested, 60 (15·5%) produced enterotoxins (Table 3): 9·9% veterinary, 12·6% dairy, and 41·7% of human strains. The latter were statistically significant ($P<0·0005$), unlike the difference between veterinary and dairy strains ($P=0·502$). Veterinary and human strains were found to produce only one kind of enterotoxin, whereas 26 (83·9%) of SE-positive dairy isolates produced one, four (12·9%) produced two (SEA/SED), and one produced three different enterotoxins (SEA/SEB/SEC). None of the strains produced SEE, and SEC was the most frequently detected.

Results of PCR for enterotoxin genes of a subset of 225 *S. aureus* strains are shown in Table 4. Overall, 115 (51·1%) strains tested positive for one or more *se* gene: 40·7% veterinary, 47·7% dairy, and 77·1% of human strains. These differences were also significant (veterinary/human, $P<0·0005$; dairy/human, $P=0·001$), except for the difference between veterinary and dairy strains ($P=0·596$). Seven distinct *se* genotypes were observed in veterinary strains, 16 in dairy strains and 13 in human strains. Twenty-six (22·6%) strains harboured only one kind of *se* gene, the remaining 89 (77·4%) isolates possessed more than one *se* gene. None of the strains harboured *see*. The majority of strains possessed the *seg* gene (86 strains or 74·8% of *se*-positive strains), to a large extent (96·5% of *seg*-positive strains) in combination

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Table 4. *Enterotoxin genotypes of veterinary, dairy, and human* S. aureus *strains*

No. of toxin genes	Genotype	No. (%) of strains			
		Veterinary	Dairy	Human	Total
Total		91 (100)	86 (100)	48 (100)	225 (100)
	se-negative	54 (59·3)	45 (52·3)	11 (22·9)	110 (48·9)
	se-positive	37 (40·7)	41 (47·7)	37 (77·1)	115 (51·1)
1	*sea*		1 (1·2)	2 (4·2)	3 (1·3)
	seb		1 (1·2)	3 (6·3)	4 (1·8)
	sec	5 (5·5)	3 (3·5)	2 (4·2)	10 (4·4)
	sed			1 (2·1)	1 (0·4)
	seg			3 (6·3)	3 (1·3)
	seh	2 (2·2)			2 (0·9)
	sei	1 (1·1)	1 (1·2)		2 (0·9)
	sej		1 (1·2)		1 (0·4)
2	*sea*/*seg*		2 (2·3)	2 (4·2)	4 (1·8)
	seb/*sec*		1 (1·2)		1 (0·4)
	seb/*sei*			2 (4·2)	2 (0·9)
	seb/*seg*		2 (2·3)		2 (0·9)
	sec/*seg*			2 (4·2)	2 (0·9)
	sed/*sej*	1 (1·1)	1 (1·2)		2 (0·9)
	seg/*sei*	22 (24·2)	17 (19·8)	9 (18·8)	48 (21·3)
	seg/*seh*			2 (4·2)	2 (0·9)
3	*sea*/*sed*/*sej*		1 (1·2)		1 (0·4)
	sea/*seg*/*sei*		1 (1·2)		1 (0·4)
	sea/*seh*/*sej*		1 (1·2)		1 (0·4)
	seb/*seg*/*sei*	2 (2·2)		2 (4·2)	4 (1·8)
	sec/*seg*/*sei*	4 (4·4)	6 (7·0)	6 (12·5)	16 (7·1)
	seg/*seh*/*sei*			1 (2·1)	1 (0·4)
4	*sec*/*seg*/*sei*/*sej*		2 (2·3)		2 (0·9)
5	*sea*/*sed*/*seg*/*sei*/*sej*		1 (1·2)		1 (0·4)

with other *se* genes. *Sec* was encountered most often in strains harbouring only a single *se* gene. The most common *se* genotype was *seg*/*sei* (48 strains or 41·7% of *se*-positive strains) followed by *sec*/*seg*/*sei* (16 strains or 13·9% of *se*-positive strains). Strains with more than three *se* genes were scarce: two dairy strains of *se* genotype *sec*/*seg*/*sei*/*sej* and one dairy strain of *se* genotype *sea*/*sed*/*seg*/*sei*/*sej*. Corresponding genes to the ELISA-based phenotypes could be detected in all cases but one dairy strain that produced SEA tested negative for the *sea* gene. Conversely three veterinary strains which were positive for *sec* did not have the corresponding phenotype, as did one *sed*-positive dairy strain, one *sec*- and one *seb*-positive human strain.

Tst was present in a total of 122 (54·2%) strains: 35 (38·5%) veterinary, 45 (52·3%) dairy, and 42 (87·5%) human strains. The difference between veterinary and dairy strains was not significant ($P=0·129$). However, significantly more *tst*-positive strains were *se*-positive than *tst*-negative strains: 71 (58·2%) of 122 *tst*-positive strains *vs.* 44 (42·7%) of 103 *tst*-negative strains ($P=0·021$). The frequency was significantly different for *seb* ($P=0·041$), *sec* ($P=0·001$), *seg*/*sei* ($P=0·014$), and *sec*/*seg*/*sei* ($P=0·035$) genotypes.

AFLP-based cluster analysis

Combined cluster analysis of 91 veterinary, 86 dairy, and 48 human strains revealed 17 clusters (A–Q) at a homology cut-off of 80% (Fig. 1). The largest cluster (D) contained 47·1% of all strains, followed by cluster I (13·8%). and cluster G (8%). The other clusters comprised 2–10 strains. Sixteen strains (7·1%) were not included in any of these clusters at this cut-off.

Nine clusters with over- or under-representation of strains with specific characteristics were identified (Table 5). However, five of these were represented

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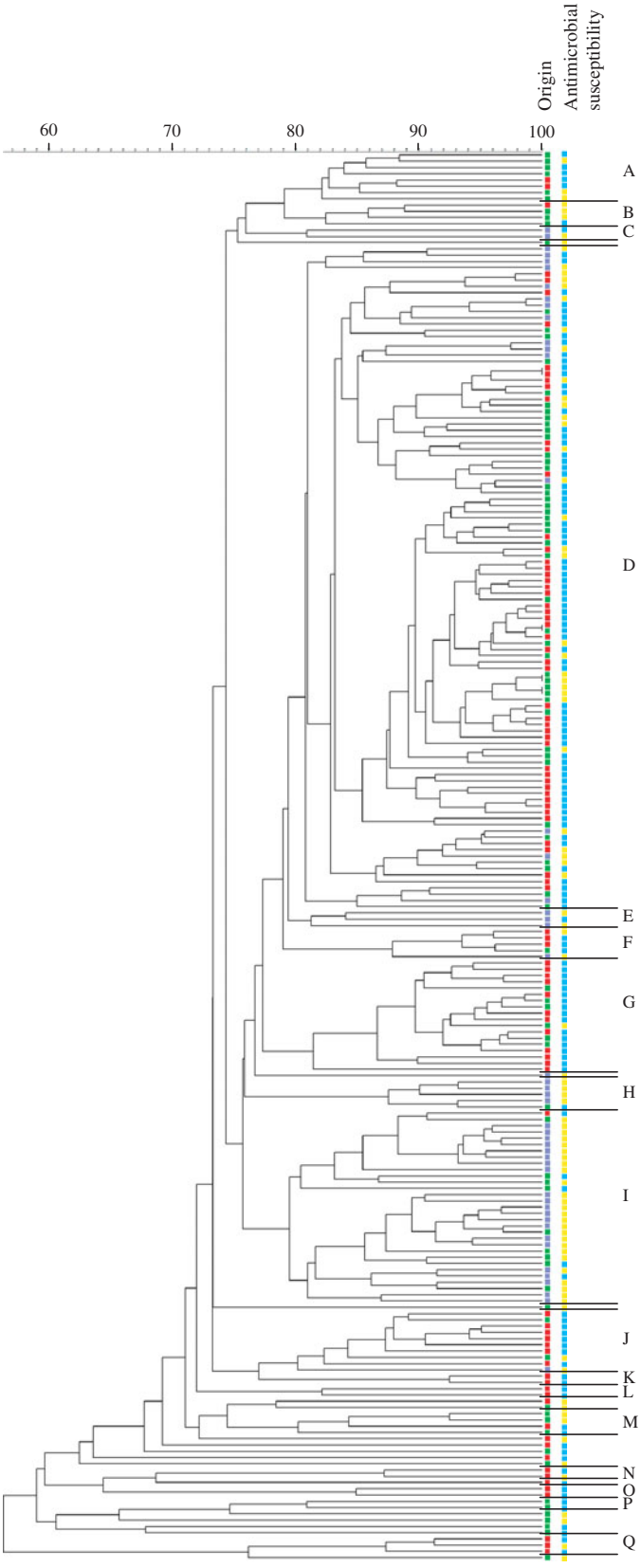

Fig. 1. Amplified fragment length polymorphism (AFLP)-based cluster analysis of veterinary, dairy and human strains. Origin and antimicrobial susceptibility of the strains are indicated by the colour of the respective boxes. Red, veterinary; green, dairy; violet, human; yellow, resistant; blue, susceptible.

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Table 5. *Statistically significant differences in the distribution of strains with specific characteristics between AFLP clusters*

Cluster	No. of isolates	Characterization of clusters			
		Over-representation	*P* value	Under-representation	*P* value
A	8			Clumping factor-positive	0·014
C	2	Human isolates	0·048		
D	106			*se*-positive	0·003
				Penicillin G-resistant	0·042
E	3	Human isolates	0·011		
F	5	*seh*	0·012	*seg*	0·019
				sei	0·040
G	18	*sei*	0·005	Egg yolk-positive	0·020
		se-positive	0·012	Clumping factor-positive	0·034
		Veterinary isolates	0·030	Antimicrobial-resistant	0·004
				Penicillin G-resistant	0·004
				Human isolates	0·028
H	5	Human isolates	0·010		
I	31	Egg yolk-positive	0·005	*sei*	0·018
		tst	0·035	*seg*/*sei*	0·023
		se-positive	<0·0005	Veterinary isolates	<0·0005
		sea	0·042		
		Antimicrobial-resistant	<0·0005		
		Penicillin G-resistant	<0·0005		
		Human isolates	<0·0005		
J	10	*sec*	0·009	Egg yolk-positive	0·017
				seg	0·046

AFLP, Amplified fragment length polymorphism.
Over- and under-representation of specific *se* genes was calculated with respect to the number of *se*-positive strains of each origin.

by only 2–8 strains. The largest cluster (D) contained veterinary, dairy and human strains. Under-representation of *se*-positive strains was mainly due to lower frequencies of this trait in dairy and veterinary strains, whereas under-representation of penicillin-susceptible strains was mainly due to lower frequencies in human and to a lesser extent in dairy strains of this cluster. Interestingly, 7/12 penicillin-resistant veterinary strains were found in this cluster. The second largest cluster (I) comprised almost exclusively dairy and human strains. Over-representation of *se*- and *sea*-positive strains was due to a higher frequency of these traits in both, dairy and human strains assembled in this cluster. By contrast, over-representation of *tst*-positive and antimicrobial-resistant strains was due to the lack of veterinary strains in this cluster. In addition, strains in cluster I had a higher frequency of antimicrobial resistance than generally observed for both origins. Under-representation of *sei*- and *seg*/*sei*-positive strains was due to the lower frequency of these genes in the strains of cluster I and to the lack of veterinary strains. Thus this cluster represented an assembly of dairy and human strains with specific traits.

The third major cluster (G) was almost exclusively made up of veterinary and dairy strains with more *se*- and *sei*-positive strains than observed for all strains of these origins. Under-representation of strains with antimicrobial resistance can be explained by a lower resistance rate in the dairy and veterinary strains of this cluster as well as the complete lack of human strains. Overall these findings suggest that the strains of cluster G might have grouped according to origin as well as specific traits. Over-representation of *sec* in cluster J comprising veterinary, dairy, and human strains was due to a higher frequency of *sec* in veterinary and dairy strains. Similarly, under-representation of *seg* could be attributed to a lower frequency of this gene in veterinary and dairy strains assembled in this cluster.

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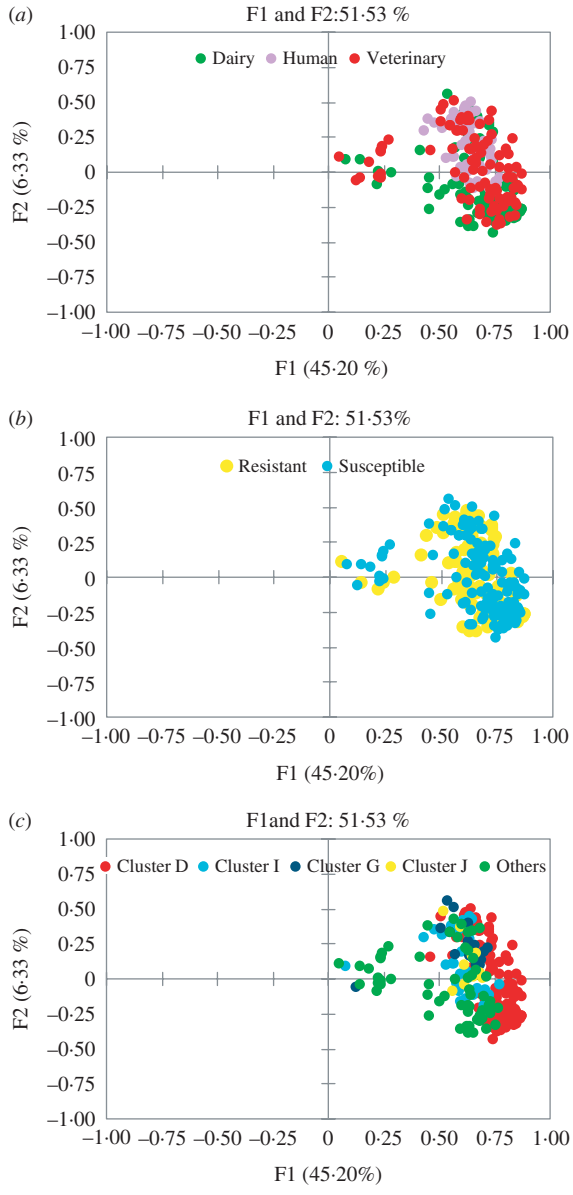

Fig. 2. Distribution of (*a*) veterinary, dairy, and human strains, (*b*) antimicrobial resistant and susceptible strains, and (*c*) strains belonging to different UPGMA clusters in a diagram defined by the first two principal components from a correlation matrix based on the binary amplified fragment length polymorphism (AFLP) data.

Representation of the AFLP results in a diagram defined by the first two principal components from a correlation matrix based on the binary AFLP data revealed close correlation between all strains (Fig. 2). Two separate groups were observed: a large group containing almost all strains and a small group containing 16 strains from veterinary and dairy origin belonging to clusters G, I, L, N, O, Q, or to no cluster. These strains did not have any specific phenotypic or genotypic traits in common. Human strains were not homogeneously distributed throughout the large group.The degree of genetic heterogeneity was similar for veterinary (0·701), dairy (0·733), and human strains (0·712).

DISCUSSION

The aim of the present study was to compare phenotypic and genotypic traits of *S. aureus* strains collected from veterinary, dairy, and human origin in order to gain information about the differences (or similarities) between strains from these sources and also between our data and those from other countries.

Clumping factor, egg-yolk reaction, and thermonuclease production

The percentage of clumping factor-positive (100%) veterinary strains was in accordance with recently published data [20, 21]. About two-fold more egg-yolk-positive veterinary strains (53·8%) were observed than reported in the literature [22, 23] but the frequency of clumping factor (94·7%) and egg-yolk reaction (43·5%) compares with other studies [24]. However, Becker *et al.* [25] reported only 44·2% of strains from quarter milk samples were positive for clumping factor. The high percentage of clumping factor-positive human strains is in agreement with other studies but here fewer egg-yolk-positive strains (85·4%) than reported by Umeki *et al.* [26] (98·5%) were detected. The overall low percentage of egg-yolk-positive strains implies that this characteristic might not be reliable for identification of the origin of *S. aureus* strains. As expected, almost all strains produced thermonuclease [27].

Antibiotic resistance of *S. aureus*

The antimicrobial resistance rates in veterinary strains, especially penicillin resistance (12·1%), are comparable with data from Switzerland [23, 28]. However, resistance rates were lower than described for Austria in 1996–1998 (20·9–28·4%) but similar to the results reported for 1987–1988 [8, 29]. Fitzgerald *et al.* [30] recovered more penicillin-resistant *S. aureus* strains from bovine intramammary infections in Ireland than reported in the present study. It is well documented that the level of penicillin resistance differs between European countries, perhaps reflecting different antimicrobial prescribing policies in each country [31]. The rates of penicillin, erythromycin and

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marbofloxacin resistance among the dairy strains accord with those from Hungary [32] while for human strains the results are in agreement with those reported in 2005 and 2006 for Austria and Germany [33, 34], but rates were lower than reported from 1996 to 2002 for Austria [10]. On the other hand, a higher percentage of penicillin- and erythromycin-resistant strains was observed for 13 European countries in 2000, with only 2% of strains being multiresistant, which is supported by the present study [35]. Differences between the data from earlier Austrian studies and from other countries might reflect changing trends in antibiotic resistance but they might also result from different sampling strategies and protocols to detect antibiotic resistance.

Production of enterotoxin A, B, C, D, E, and presence of *se* genes and *tst*

The frequency of enterotoxin-producing veterinary strains (9·9%) is close to data published for California in 2003 [21], and for enterotoxigenic dairy strains (12·6%), this accords with results for France in 1997 [36]. SEC was most frequently produced by veterinary and dairy strains and this is supported by other studies [37, 38]. Enterotoxin-producing human strains (41·7%) were considerably more frequent than veterinary (9·9%) and dairy strains (12·6%) and similar to recently reported rates [39]. In other studies the prevalence of SEs and also the frequency of each SE were highly variable [21, 38] and this might be explained by variation in assays and culture conditions used to detect enterotoxins. The absence of SEE and the *see* gene accords with the results of others [39, 40].

PCR assays showed good correlation with the expression of enterotoxin proteins detected by ELISA but PCR identified a higher number of enterotoxin-positive strains. The finding that in one strain SEA could not be detected by PCR might be explained by sequence variation in the *sea* gene or a novel toxin cross-reacting in the ELISA [16, 37]. The most common combination within all strains was *seg*/*sei* followed by *sec*/*seg*/*sei*. Similar results were found in other European countries [41, 42]. In animal-associated strains *sea*, *seb*, *see*, and *seh* were absent or rarely present as commonly observed [21, 30]. A small percentage of human strains harboured the classical enterotoxin genes *sea*, *seb* or *sec*, but *seg* and *sei* were found in 50% and 41·7% of human strains, respectively. The function of the recently described toxin genes as pathogenic agents remains unclear but *S. aureus* strains involved in food-poisoning outbreaks lacking classical enterotoxins but expressing *seg*, *sei*, and *sej* have been described [16].

The proposed coexistence of toxin genes *sed* and *sej* on a common plasmid was not supported by our data [43]. Similar results were found by others [40, 44]. Coexistence of *seg* and *sei* on the *egc* enterotoxin cluster in *S. aureus* is also controversial [34, 37, 40, 44]. In the present study *seg* was detected in 84·9% of all cases together with *sei*. However, sequence variation might also account for these results.

The frequency of *tst* among veterinary strains (38·5%) was lower than reported for Switzerland (64·7%) [38] but the percentage of *tst*-positive dairy strains (52·3%) was slightly higher than found recently in Norway [37]. Contrary to studies performed in other countries which have reported 6·5–40% *tst*-positive human strains, we found most (87·5%) human strains were *tst* positive [34, 45, 46]. An alternative PCR method was performed on selected strains and confirmed the results, thus non-specific PCR amplification could be ruled out (data not shown) [17]. In our study 20·5% of all *tst*-positive strains were positive for *sec* and only 2·6% of *sec*-positive strains carried no gene for *tst*. Significant correlation between SEC and TSST has been reported for bovine strains [30, 38], perhaps due to the fact that both genes are located on the bovine staphylococcal pathogenicity island (SaPIbov), a mobile genetic element [47]. Our investigations showed that of nine veterinary strains carrying the gene for *sec*, seven also carried *tst*. In addition, only one of 12 *sec*-positive dairy strains had no *tst* gene. The rare co-occurrence of *sec* and *tst* genes in human strains has been reported [41, 46]. This contrasts with our finding of 80% of *sec*-positive human strains also carrying *tst*.

AFLP-based cluster analysis

Clusters D, G, I, and J comprised almost 70% of all strains and contained strains with different frequencies of *se* genes or antimicrobial resistance than observed for all strains of each origin. In addition, two of these clusters grouped strains according to origin, since in both cases mainly two niches were represented in the cluster. Recently published AFLP analysis of *S. aureus* strains from human and animal origin also revealed a tendency to cluster according to origin whereas single enzyme AFLP of human, bovine, ovine, and food-related sources revealed a tendency to cluster according to *se* genotypes [39, 45].

In conclusion, many of the phenotypic and genotypic traits of *S. aureus* strains from veterinary, dairy, and human origin were similar to those reported from other European countries. However, marked differences were observed between strains from different origins for antimicrobial resistance, presence of *se* genes and *tst* gene. An outstanding finding was the high percentage of *tst*-positive human strains. Since *S. aureus* strains characterized here were collected from human wound infections only, further research should focus on comparing the percentage of *tst*-positive human strains from different clinical settings and from healthy individuals. Standardized methods for characterization of *S. aureus* strains would greatly improve the comparison of data from different ecological niches and different countries. The European Antimicrobial Resistance Surveillance System (EARSS) for human *S. aureus* strains was established in 1998 with the aim of obtaining comparable and reliable data for public health. Extension of this programme to the monitoring of toxins and inclusion of veterinary strains is worthy of consideration.

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DECLARATION OF INTEREST

None.

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