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Epidemiology and characterization of *Staphylococcus epidermidis* isolates from humans, raw bovine milk and a dairy plant

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SUMMARY

Geographically related *Staphylococcus epidermidis* isolates from human patients ($n=30$), dairy farms (farmers and individual raw milk from cattle, $n=36$) and a dairy plant ($n=55$) were examined for epidemiological relatedness by pulsed-field gel electrophoresis and, using *in vitro* methods, for the ability to produce biofilm and antimicrobial resistance. Methicillin-resistant isolates (MRSE) were also identified and characterized. Isolates from farmers and dairy cattle were found to be genetically related, while isolates from human patients were highly diverse. Some dairy plant isolates (18·2%) were closely related to those from dairy farms. Biofilm production and resistance to antimicrobial agents were most typical for isolates from human patients, of which 76·7% were MRSE. Methicillin resistance was also widespread in farm-related isolates (61·1%). This study indicates the possible transmission of *S. epidermidis* between cattle and farmers. Dairy products were not proven to be an important source of either human infections or methicillin-resistant strains.

Key words: Antibiotic resistance, epidemiology, pulsed-field gel electrophoresis (PFGE), *Staphylococcus epidermidis*.

INTRODUCTION

Unlike *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS) have not been given significant attention for a long time. In recent times, however, their involvement in various types of infections has been increasingly recognized [1]. Accordingly, *Staphylococcus epidermidis*, one of the most important members of CoNS has been described not only as a part of normal microbiota but also as a causative agent of various infections in humans [1]. Moreover, it has been shown that a novel genomic island encoding for multiple phenol-soluble modulins, a potential virulence factor, may contribute to the evolution of this species from a commensal pathogen to a more aggressive pathogen [2]. *S. epidermidis* has often been described in humans as one of the most important opportunistic pathogens of the genus *Staphylococcus*, causing infections in immunocompromised individuals [3]. Its clinical importance in animals has been recognized mainly in connection with mastitis [4].

Biofilm formation is considered an important factor involved in the pathogenesis of *S. epidermidis*.

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Table 1*a*. Staphylococcus epidermidis *isolates included in the current study*

Origin of isolates	No. of isolates*	Type of sample
Human patients†		
Hospitalized patients	15	Various samples
Outpatients	15	Various samples
Dairy farms‡		
Dairy cattle§	22	Individual cow raw milk
Farmers¶	14	Nasal and rectal swabs
Dairy plant‖		
Final milk products	47	Pasteurized (8), UHT (5) and dried (9) milk; butter (10); fermented milk products (15)
Surface scrapings	8	Pasteurizer; piping and tanks for pasteurized, UHT and dried milk; balance tanks; processing equipment for fermented milk products and butter

UHT, Ultra high temperature.
* One isolate per sample.
† Patients associated with one hospital: (i) hospitalized patients, (ii) patients previously hospitalized and undergoing ambulatory care treatment in the same hospital (outpatients).
‡ Six farms were monitored.
§ Individual raw milk from randomly selected dairy cattle exhibiting no clinical signs of mastitis.
¶ Nasal and rectal swabs from randomly selected healthy farmers who milked the cows.
‖ Samples from one dairy (the only dairy in the region) which collects and processes milk from the six monitored farms (numbers of isolates from the specific milk products are given in parentheses).

In human medicine, biofilm-producing *S. epidermidis* strains have become well recognized for their role in in-dwelling or implanted device-related infections [5]. Biofilm production was also determined in various staphylococci, including *S. epidermidis*, isolated from bovine mastitis [4]. In addition, staphylococci (including *S. epidermidis*) have been described as bacteria which may attach, form biofilms and survive on the contact surfaces in both the milk and meat-processing industries [6, 7]. Their attachment to food contact surfaces in dairy plants and subsequent biofilm formation pose a risk for secondary contamination of milk and milk products [6].

In recent times, *S. epidermidis* has been characterized by an increasing antimicrobial resistance rate [8]. Spread of multi-resistant strains of methicillin-resistant *S. epidermidis* (MRSE) represents a serious problem. The occurrence of MRSE has been mainly monitored in humans [1, 9], but its emergence in bovine milk has also been recently reported [10]. DeAraujo *et al*. [11] reported an association between multi-resistance (methicillin resistance) and biofilm production in *S. epidermidis* and speculated that increased genetic exchange in the biofilm environment may contribute to the multi-resistance phenotype. Because staphylococci are able to form biofilm on inert materials used in the food-processing industry [7], foodstuffs cannot be excluded as one of the possible sources of multi-resistant *S. epidermidis* strains.

The aim of this study was to assess the epidemiological relatedness in *S. epidermidis* isolates originating from different sources (humans, animals, foodstuffs, environment) by pulsed-field gel electrophoresis (PFGE), and further to analyse the isolates for their ability to form biofilm and their antimicrobial resistance profiles (including identification and characterization of MRSE isolates).

METHODS

S. epidermidis isolates and growth conditions

A total of 121 *S. epidermidis* isolates, obtained during the period 2006–2008 from different sources within one selected district in the Czech Republic, were analysed in our study (Table 1*a*). The isolates originated from human patients ($n=30$), dairy farms ($n=36$), and from a dairy plant ($n=55$) and were routinely grown at 37 °C on blood agar (Blood Agar Base No. 2, HiMedia, India) containing 5% sheep blood.

Sample preparation and isolation of *S. epidermidis*

Surface scrapings from a dairy plant were immersed in 10 ml phosphate-buffered saline (PBS) containing 0·1% peptone and processed according to ISO standard EN ISO 6887-1. Sampling of milk and milk products and subsequent processing of the samples

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Table 1*b*. Staphylococcus epidermidis *isolates from human patients*

Disease/diagnosis	Site of isolation	No. of isolates
Hospitalized patients		
Post-operative wound infection	Wound	1* (1/1)
Cannula-associated infection	Cannula/blood	7* (4/5)
Pneumonia	Blood/BAL	4 (0/3)
Urinary infection	Urine	2 (0/1)
Non-bacterial infection	Cannula	1 (0/0)
Outpatients		
Post-operative wound infection	Indwelling device	6* (6/6)
Sinusitis	Puncture	1 (0/1)
Non-infectious	Various†	8 (1/6)

BAL, Bronchoalveolar lavage.
Asterisks indicate the isolates which were confirmed as causative agents of the specific disease. Numbers of biofilm-positive/methicillin-resistant isolates as determined in this study are shown in parentheses.
† In-dwelling devices and skin, throat and rectal swabs.

were done according to standards EN ISO 6887-2 and EN ISO 6888-3. One hundred microlitres of the analytical sample were then selectively cultivated on both Baird–Parker and Kranep agars (Merck, Germany) at 37 °C for 48 h. Samples from humans (Table 1*b*) were inoculated onto blood agar (Becton Dickinson, USA) and subsequently incubated at 37 °C for 24 h. The isolates were identified by standard microbiological procedures using STAPHYtest 24 (Pliva-Lachema, Czech Republic) and a Vitek 2 automated system (bioMérieux, France). The presumptive identification of *S. epidermidis* was confirmed by PCR as described below.

Confirmation of *S. epidermidis* and detection of the *ica* operon

SE705-1 and SE705-2 primers [12] and icaAB-F and icaAB-R primers [13], respectively, were used to amplify a species-specific SE705 sequence and a part of the *ica* operon, an essential factor involved in biofilm formation. The sequence of 16S-rDNA amplified with UNB1 and UNB2 primers [14] was included as an internal positive control and the PCR reaction was performed as described previously [14]. The expected sizes of the PCR products were 124, 370 and 546 bp for SE705, 16S rDNA and *ica* amplicons, respectively. The biofilm-positive *S. epidermidis* CCM 7221 and the biofilm-negative *S. epidermidis* ATCC 12228 strains were used as positive and negative controls, respectively.

PFGE

The isolation of DNA was performed according to Linhardt *et al*. [15] as modified by Pantucek *et al*. [16]. DNA was digested with 8 U *Sma*I (New England BioLabs, UK) at 25 °C for 18 h. The restriction fragments were separated in 1·2% PFGE Grade Agarose III gel (Amresco Inc., USA) in TBE buffer. Electrophoresis was carried out on the CHEF-DR III System (Bio-Rad, USA) with a voltage of 5·5 V/cm for 27·5 h with an initial switch time of 1 s, increasing to 35 s. Restriction endonuclease patterns (PFGE types) were analysed with Gel Compar software (Applied Maths, Belgium) using the Dice coefficient and the UPGMA algorithm with 1% tolerance and 0·5% optimization settings.

In vitro methods for detection of biofilm formation

The ability for biofilm formation was tested in polystyrene microtitration plates for tissue cultures (Becton Dickinson Labware, France) according to Cucarella *et al*. [17] and on Congo Red agar (CRA) according to Arciola *et al*. [18]. The biofilm-positive *S. epidermidis* CCM 7221 and the biofilm-negative *S. epidermidis* ATCC 12228 strains were used as positive and negative controls, respectively.

Antimicrobial susceptibility testing

The isolates were tested for their susceptibility to selected antimicrobial agents by determination of minimum inhibitory concentrations (MICs) using the broth microdilution method, according to the approved standard of the Clinical and Laboratory Standards Institute (CLSI) (document M7-A7). Tested antimicrobial agents were benzylpenicillin (PEN), oxacillin (OXA), chloramphenicol (CMP), tetracycline (TET), trimethoprim–sulphamethoxazole (COT) at a ratio of 1:19, erythromycin (ERY), clindamycin (CLI), ciprofloxacin (CIP), gentamicin (GEN), teicoplanin (TEI) and vancomycin (VAN). The test was performed using commercially prepared MIC panels (ST *Staphylococcus* spp.; Trios, Czech Republic). The MIC interpretation criteria were based on guidelines from the CLSI (document M100-S16). *S. aureus* ATCC 25923 served as a reference strain for quality control purposes.

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Table 2. *List of the primers used in* SCC*mec typing*

Primer				
Name	Specification	Amplified sequence (size)	Specificity (SCC*mec* type)	Ref.
CIF	CIF2 F2 CIF2 R2	Downstream of the *pls* gene (495 bp)	I	[19]
KDP	KDP F1 KDP R1	Internal to the *kdp* operon (284 bp)	II	[19]
DCS	DCS F2 DCS R1	Internal to the *dcs* region (342 bp)	I, II, IV, VI	[19]
RIF	RIF4 F3 RIF4 R9	Between pI258 and Tn*554* (243 bp)	III	[19]
ccrB	ccrB2 F2 ccrB2 R2	*ccr* complex (311 bp)	II, IV	[22]
ccrC	ccrC F2 ccrC R2	*ccr* complex (449 bp)	V	[22]

Identification and characterization of MRSE isolates

Isolates with MICs $\geqslant 0{\cdot}5\ \mu$g/ml for OXA were tested for the presence of the *mec*A gene by PCR using MECA-P4 and MECA-P7 primers [19], HotStarTaq Master Mix kit (Qiagen, Germany) under the following conditions: initial denaturation at 94 °C for 15 min. followed by 30 cycles of denaturation at 94 °C for 1 min. annealing at 55 °C for 1 min. and extension at 72 °C for 1 min with final extension at 72 °C for 5 min. *S. aureus* ATCC 33591 and *S. epidermidis* ATCC 12228 strains served as positive and negative controls, respectively. In the *mec*A-positive isolates, the staphylococcal chromosomal cassette carrying the *mec*A gene (SCC*mec*) was characterized by PCR typing using six pairs of primers (Table 2). Each primer pair was tested in a single PCR reaction. Two microlitres of purified DNA were added to an 18 μl PCR mixture and the final mixture contained the following: 0·5 μM concentrations of each primer, 217 μM each dNTP (Invitek, Germany), 2·5 mM $MgCl_2$, 1× DyNAzyme II PCR buffer and 0·8 U DyNAzyme II DNA polymerase (Finnzymes, Finland). The PCR amplification was performed in a PTC-0220 DNA Engine Dyad Thermal Cycler (Bio-Rad Laboratories Inc., USA) under the following conditions: initial denaturation at 94 °C for 5 min. followed by 35 cycles of denaturation at 94 °C for 30 s. annealing at 56 °C for 1 min. and extension at 72 °C for 1·5 min with final extension at 72 °C for 5 min. *S. aureus* strains NCTC 10442 (SCC*mec* type I), N315 (SCC*mec* type II), 85/2087 (SCC*mec* type III), JCSC 4744 (SCC*mec* type IV) and WIS (SCC*mec* type V), kindly supplied by Teruyo Ito (Juntendo University, Tokyo, Japan), and *S. aureus* strain HDE288 (SCC*mec* type VI) [20], kindly supplied by Herminia de Lencastre (ITQB-Universidade Nova de Lisboa, Portugal) were used as positive controls.

RESULTS

PFGE types and genetic relatedness of *S. epidermidis* isolates

Of the 121 *S. epidermidis* isolates, a total of 58 PFGE types were observed: 14 in the isolates ($n=15$) from outpatients, 13 in the isolates ($n=15$) from hospitalized patients, 14 in the isolates ($n=14$) from farmers, eight in the isolates ($n=22$) from dairy cattle (individual raw milk), 16 in the isolates ($n=47$) from final milk products, and six in the isolates ($n=8$) from surface scrapings. While a high diversity was observed in the isolates of human origin, the isolates from dairy cattle and final milk products were more uniform. Discrimination of the isolates by using a cut-off value of 79%, as previously proposed [21], revealed eight clusters comprising 36·4% ($n=44$) of the isolates (Fig. 1). In our study, clumps of isolates in which more than one PFGE type were found were considered as clusters. With the exception of one cluster, in which eight isolates from the dairy plant and 14 isolates from cattle were found, no substantial number of the isolates clustered together at a genetic similarity level of $\geqslant$79%. We also observed 13 clumps of isolates (not included in the clusters) in which only one of the PFGE types was found.

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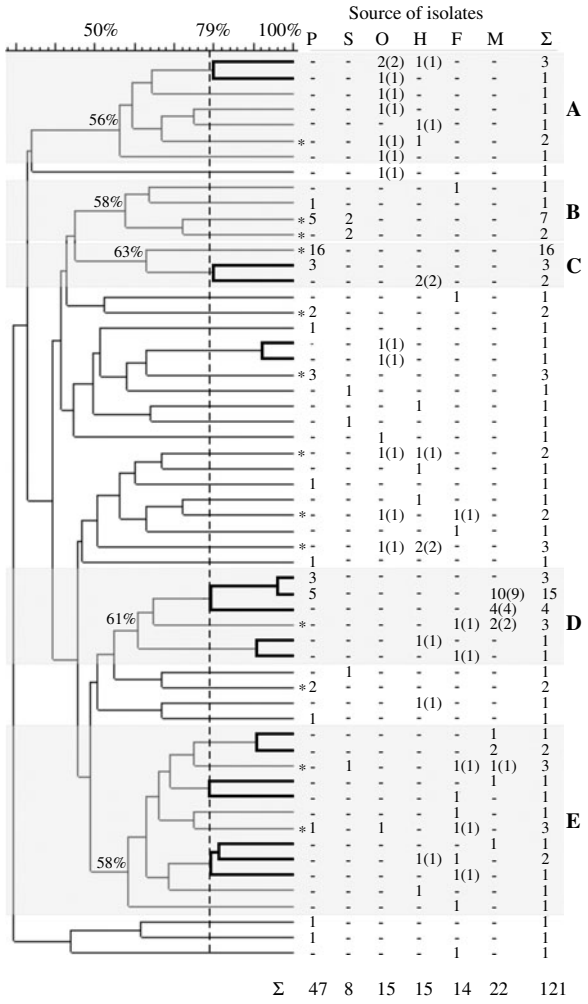

Fig. 1. Dendrogram of genetic similarity in 58 PFGE types observed in *Staphylococcus epidermidis* isolates. Numbers of the isolates of the same PFGE type are shown (numbers of the MRSE isolates are indicated in parentheses). Eight clusters are indicated with bold lines. Asterisks indicate 13 clumps of the isolates which were not included in the clusters and were characterized by a unique PFGE type. Letters A, B, C, D and E indicate five different groups of the isolates (P, final milk products; S, surface scrapings from the dairy; O, outpatients; H, hospitalized patients; F, farmers; M, individual raw milk from dairy cattle).

Similarly to the clusters, these clumps consisted of low numbers of isolates (mostly 2–3), except for the 16 isolates taken from the dairy plant. In an attempt to illustrate the genetic relatedness of the isolates with respect to their origin, five groups (A–E) comprising a majority of the isolates (87; 71·9%) are indicated in Fig. 1. Ten (33·3%) isolates from human patients formed a distinct group (A) and could be separated from all the dairy plant and farm-related isolates at genetic similarity levels of 29% or 33%. In the isolates from human patients, no substantial association was observed between their involvement in the infection and a particular PFGE group/cluster. Groups B and C were most specific for the dairy plant-related isolates and comprised 52·7% ($n=29$) of those isolates. All the isolates from cattle and a majority ($n=9$; 64·3%) of the isolates from farmers were found in groups D and E. Ten (18·2%) isolates from the dairy plant were also found in these two groups and clustered together or even shared the same PFGE type with some of the isolates identified on the farms.

Prevalence of biofilm-positive *S. epidermidis* isolates

Isolates which were positive using at least one of the tests (21 isolates) were considered as biofilm-positive. A correlation in the tests using microtitration plates (MP), CRA and *ica*-specific PCR (*ica*-PCR) was observed since 16/21 biofilm-positive isolates were positive by all these testing methods. From the remaining five biofilm-positive isolates, one isolate was positive only by MP test, one isolate was positive by both the CRA test and *ica*-PCR, and three isolates were positive only by *ica*-PCR (Table 3). The highest prevalence of biofilm-positive isolates was observed in the isolates of human origin (46·7%, 33·3% and 28·6% of the isolates from outpatients, hospitalized patients and farmers, respectively). Most of the biofilm-positive isolates from human patients were involved in in-dwelling device-related infections (Table 1*b*). Biofilm positivity was less frequently observed in isolates from final milk products (8·5%) and individual raw milk (4·5%). No biofilm-positive isolate was detected on the surfaces tested in the dairy.

Antimicrobial resistance phenotypes in *S. epidermidis* isolates

Numbers of isolates resistant to individual antimicrobial agents tested in this study are shown in Table 4. In general, antimicrobial resistance was most prevalent in the isolates from human patients regardless of whether these isolates were involved in the infection or not. The isolates from farmers were less frequently found to be resistant. A high prevalence of resistance to some particular antimicrobial agents was also observed in the isolates from dairy cattle (individual raw milk) and the dairy plant. Resistance to PEN was generally frequent in all the isolates and resistance to OXA was often detected in the isolates from humans and cattle. Furthermore, resistance to COT, ERY, CLI, CIP and GEN was more

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Table 3. *Numbers of biofilm-positive and methicillin-resistant* Staphylococcus epidermidis *isolates*

	Biofilm				
Source	MP*	CRA†	*ica*‡	MRSE§	Phenotype of resistance of MRSE¶
Hospitalized patients	4	4	5	10	ERY (7); CIP (7); COT (6); GEN (3); CLI (2); CMP (1); TET (1)
Outpatients	5	6	7	13	ERY (11); COT (10); CIP (9); GEN (9); CLI (7); CMP (3); TET (1)
Farmers	3	3	4	6	COT (5); TET (5); ERY (4); CLI (3); CIP (2); GEN (1); CMP (1)
Dairy cattle	1	1	1	16	TET (15); COT (9); ERY (1); CLI (1)
Final milk products	4	3	3	—	—
Surface scrapings	—	—	—	—	—
Total	17	17	20	45	

MP, Microtitration plate; CRA, Congo Red agar; MRSE, methicillin-resistant *Staphylococcus epidermidis*.
* Biofilm development in microtitrate plates.
† Detection of exopolysaccharides on CRA.
‡ PCR positivity for *ica*.
§ Numbers of MRSE isolates positive for *mec*A by PCR.
¶ Numbers of MRSE isolates (in parentheses) resistant (or intermediately resistant) to the indicated antimicrobial agents (all the MRSE isolates were resistant to OXA and PEN).

Table 4. *Numbers of isolates resistant* (*intermediately resistant*) *to individual antimicrobial agents*

		Antimicrobial drugs										
Origin of isolates	No.*	PEN	OXA	CMP	TET	COT	ERY	CLI	CIP	GEN	TEI	VAN
Hospitalized patients	15	12 (0)	10 (0)	0 (1)	1 (0)	6 (0)	7 (0)	2 (0)	8 (0)	3 (0)	0 (0)	0 (0)
Outpatients	15	15 (0)	13 (0)	3 (0)	0 (1)	10 (0)	12 (0)	7 (0)	9 (0)	9 (0)	0 (0)	0 (0)
Farmers	14	6 (1)	6 (0)	1 (0)	4 (1)	5 (0)	5 (0)	3 (0)	2 (0)	1 (0)	0 (0)	0 (0)
Dairy cattle	22	21 (0)	16 (0)	0 (0)	16 (0)	5 (4)	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Final milk products	47	20 (0)	0 (0)	2 (0)	2 (0)	2 (6)	36 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Surface scrapings	8	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	121	76 (1)	45 (0)	6 (1)	23 (2)	28 (10)	64 (0)	13 (0)	19 (0)	13 (0)	0 (0)	0 (0)
Total, %	100	62·81 (0·83)	37·19 (0·00)	4·96 (0·83)	19·01 (1·65)	23·14 (8·26)	52·89 (0·00)	10·74 (0·00)	15·70 (0·00)	10·74 (0·00)	0·00 (0·00)	0·00 (0·00)

PEN, Benzylpenicillin; OXA, oxacillin; CMP, chloramphenicol; TET, tetracycline; COT, trimethoprim–sulphamethoxazole; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; GEN, gentamicin; TEI, teicoplanin; VAN, vancomycin.
* Number of isolates.

characteristic for the isolates from human patients while resistance to TET was typical for isolates collected from cattle (resistance to TET was also confirmed in four isolates from farmers). Although the resistance of dairy plant-related isolates was generally low (with the exception of PEN), a high number of these isolates were resistant to ERY.

Prevalence of MRSE isolates and SCC*mec* typing

Forty-five (37·2%) isolates with MICs $\geqslant 0{\cdot}5\,\mu$g/ml for OXA were positive for *mec*A by PCR and thus confirmed as MRSE. While a high prevalence of MRSE was observed in the isolates from humans and cattle (individual raw milk), no MRSE isolate was

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Table 5. *Coding system for the* SCC*mec profiles*

First digit of numeric code			Second digit of numeric code		
CIF	KDP	DCS	RIF	ccrC	ccrB
4	2	1	4	2	1

Numbers assigned to each sequence amplified by the indicated primers are shown. Numbers assigned to the sequences amplified by CIF, KDP and DCS primers are added, and numbers assigned to the sequences amplified by RIF, ccrB and ccrC primers are added. The two resulting digits are joined as a numeric code. For example, if all the sequences were amplified the digits 7 and 7 would be generated and then combined into the numeric code 77.

found in the dairy plant-related samples (Table 3). The highest proportion of MRSE was observed in the isolates from outpatients (86·7%), followed by the isolates from cattle (72·7%), hospitalized patients (66·7%) and farmers (42·9%). Besides the resistance to OXA and PEN, resistance to ERY dominated in the MRSE isolates from human patients while resistance to TET prevailed in the MRSE isolates from cattle. The occurrence of ERY and TET resistance was similar (4:5) in the MRSE isolates from farmers (Table 3).

Using the primers listed in Table 2, we observed a high variability of SCC*mec* in the MRSE isolates. However, a majority of these isolates ($n=40$) were nontypable according to previous studies [19, 22]. In other words, the PCR patterns of the non-typable isolates differed from those suggested in those studies to be specific for SCC*mec* types I–VI. The occurrence of two or more sequences, each specific for a different SCC*mec* type, in a single isolate was observed quite often. A similar finding was also observed in four *S. aureus* control strains. The PCR products from one selected isolate (SEP1692), which was positive for CIF, DCS, RIF, ccrB and ccrC primers, as well as the control strains, were checked for their specificity by sequencing (Genex CZ, Czech Republic). Due to the high variability of SCC*mec* (12 different PCR patterns) and because no substantial correlation between particular sequences was observed, we determined the SCC*mec* profiles using a numeric coding system as shown in Table 5. The prevalence of different SCC*mec* profiles (Table 6*a*) and the PCR patterns detected in the control strains (Table 6*b*) are also shown. While a high variability of SCC*mec* was found in the isolates of human origin, all of the MRSE isolates from dairy cattle had a uniform SCC*mec* profile. In general, no substantial association between particular SCC*mec* and PFGE types was observed. The same PFGE type was found in 9/18 MRSE isolates of SCC*mec* profile 41, but four different PFGE types were found in the remaining nine isolates.

DISCUSSION

The intention of our study was to expand current knowledge regarding the epidemiology, virulence potential and strain diversity of *S. epidermidis*. Characterization of the isolates by PFGE revealed a high diversity, as also observed by other authors [23, 24]. While a variety of studies (e.g. [25]), have suggested the possible transmission of *S. aureus* between humans and animals, very little is known about whether such a transmission may occur with *S. epidermidis*. Thorberg *et al*. [23], analysed *S. epidermidis* from cows' milk and farmers' skin, and demonstrated that bovine *S. epidermidis* mastitis may emanate from the farmers. In our study, two PFGE types which were identified in two isolates from farmers were also found in three isolates from cattle (one isolate from farmers and two isolates from cattle both sharing the PFGE and SCC*mec* profile). This finding together with a certain degree of genetic similarity observed between the isolates from these two hosts may indicate that close contact between humans and animals could be a risk factor involved in the transmission of *S. epidermidis*. Due to the fact that *S. epidermidis* belongs to the normal flora of humans, farmers could be a more probable source of infection.

A high diversity was found in the isolates from human patients, even though these patients were associated with a single hospital. This diversity was observed both in isolates involved in the infection and those not involved. Within a single hospital, clonal and non-clonal distribution of *S. epidermidis* has already been reported [26]. Ten isolates (18·2%) from the dairy plant were closely related to some of the isolates from farms. Moreover, five of the isolates shared the same PFGE type with ten isolates from raw bovine milk (Fig. 1; group D). Therefore, the raw milk appeared to be a potential source of contamination. This finding is in agreement with a similar study dealing with *S. aureus* [27] in which 21% of the isolates from milk products were related to the isolates in raw milk. However, most of the isolates from the dairy plant were distinct from the farm-related isolates, and therefore bacterial contamination is

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Table 6*a*. *Distribution of different* SCC*mec profiles in MRSE isolates*

Source	SCC*mec* profile (numeric codes)											
	7	10*	11†	13	41	43	45	46	51	54	55	58
Hospitalized patients	—	1	—	—	1	—	3	1	1	—	3	—
Outpatients	—	1	2	1	—	—	1	—	3	1	3	1
Farmers	1	—	1	—	1	1	—	—	2	—	—	—
Dairy cattle	—	—	—	—	16	—	—	—	—	—	—	—
Total	1	2	3	1	18	1	4	1	6	1	6	1

* Numeric code 10 corresponds to SCC*mec* type VI (isolates positive only for DCS primers).
† Numeric code 11 corresponds to SCC*mec* type IV (isolates positive only for DCS and ccrB primers).

Table 6*b*. *PCR patterns observed in the control strains*

Strain (SCC*mec* type)	PCR patterns		Numeric code†
	Expected*	Observed	
NCTC 10442 (I)	CIF, DCS	CIF, DCS	50
N315 (II)	KDP, DCS, ccrB	CIF, KDP, DCS, ccrB	71
85/2087 (III)	RIF	CIF, RIF, ccrC	46
JCSC 4744 (IV)	DCS, ccrB	CIF, DCS, ccrB	51
WIS (V)	ccrC	RIF, ccrC	06
HDE 288 (VI)	DCS	DCS	10

* According to Oliveira & de Lencastre [19] and Milheirico *et al*. [22].
† Determined for the PCR patterns observed in this study.

probably of multiple origins. Furthermore, 16 isolates (Fig. 1; group C) were of the same PFGE type, which indicates a common source of contamination in the dairy plant environment.

Biofilm production has been recognized as a typical virulence feature of *S. epidermidis* strains involved in implanted device-related human infections [5]. In the current study, biofilm production was a characteristic of isolates from implant infections (Table 1*b*). On the other hand, Boynukara *et al*. [28] found that 60% of CoNS (including *S. epidermidis*) obtained from various human clinical specimens were slime producers. Presterl *et al*. [29] reported a high prevalence of biofilm-positive *S. epidermidis* isolates not only in patients with implant infections (86·4%) but also in those with transient bacteraemia (88·8%). However, whether biofilm production contributes to the development of various types of *S. epidermidis* infections in humans (as demonstrated for *S. aureus* in relation to chronic rhinosinusitis [30]) remains to be elucidated. Healthy farmers examined in our study were also carriers of biofilm-positive *S. epidermidis*. Similarly, it has been reported that 30% of healthy medical students harboured biofilm-forming *S. epidermidis* [31] and that 76·9% and 20% of *S. epidermidis* isolates from the skin of healthy volunteers and milk of healthy women, respectively, were biofilm producers [29, 32].

The occurrence of biofilm-positive isolates in the individual raw milk sampled in the current study was sporadic (1/22, 4·5%). Similarly, a low number (4/55, 7·3%) of biofilm-positive isolates was observed in the dairy plant. A low prevalence of biofilm production in food-related *S. epidermidis* isolates has also been described in other studies [7, 14]. This may indicate that *S. epidermidis* strains isolated from food or food-processing environments are less invasive than clinical strains.

Antimicrobial resistance phenotypes generally differed between isolates of different origin. While a high prevalence (72·7%) of TET resistance was observed in the isolates from raw milk, resistance to ERY dominated (70·9%) in the dairy plant-related isolates. This confirms the finding by PFGE that the dairy farms examined in our study are not the main source of contamination. Sawant *et al*. [10] reported a relatively high (37·8%) prevalence of ERY resistance in *S. epidermidis* from raw bovine milk. This, together with a

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54·5% prevalence of resistance to ERY found in the human isolates examined in our study, indicates that milk from other dairy farms as well as personnel should also be considered as possible sources of contamination.

Similarly to a previous observation [33], a high prevalence of resistance to PEN, OXA, ERY, CIP and COT was observed in the isolates from human patients. This finding correlated with a high number of MRSE isolates (23/30, 76·7%) found in human patients. The phenotype of antimicrobial resistance in the MRSE isolates (Table 3) was comparable to those described previously [34]. In addition to those in patients, MRSE isolates (6/14, 42·9%) expressing an increased level of antimicrobial resistance also occurred in healthy farmers. An increased incidence of methicillin resistance has already been reported in CoNS from healthy carriers [35]. This could be a warning signal, since these bacteria can serve as reservoirs of resistance determinants in the community [36]. In our study, one isolate collected from a farmer had the same PFGE and SCC*mec* profile as one of the isolates from an outpatient that had caused a postoperative wound infection. Another important finding in the current study is the fact that 72·7% (16/22) of the bovine milk isolates were identified as MRSE. This prevalence was higher than those recently described [10, 37], where 29% and 32·4%, respectively, of isolates in *S. epidermidis* from bovine milk were identified as MRSE.

No general correlation between the presence of the *mec*A gene and biofilm positivity was observed. The *mec*A gene was found in 13/21 biofilm-positive isolates. Moreover, *mec*A was also detected in 32 biofilm-negative isolates. However, almost all of the human biofilm-positive isolates (13/16) were also *mec*A-positive (only one biofilm-positive isolate from a hospitalized patient and two from farmers were *mec*A-negative). This indicates a high association between biofilm production and multi-resistance (methicillin resistance) in human *S. epidermidis* strains. Similarly, a significant association between the presence of *ica* genes and multiple resistance in human *S. epidermidis* was observed by Montanaro *et al*. [38]. An association of biofilm production and methicillin resistance was not observed in the current study in the non-human biofilm-forming isolates, since all of them ($n=5$) were *mec*A-negative. Nevertheless, a high correlation between the presence of *ica* genes and methicillin resistance has already been determined in *S. epidermidis* from bovine milk [10].

Besides the high variability of SCC*mec*, the majority (88·9%) of the MRSE isolates could not be assigned to any of the known SCC*mec* types. This might be explained by the fact that the SCC*mec* typing used in the current work was originally designed for *S. aureus* [19]; however, the four *S. aureus* control strains tested in our study were also non-typable. It also should be mentioned that our PCR conditions differed from those originally described [19]. On the other hand, Machado *et al*. [39], using the same typing strategy, had successfully typed SCC*mec* in 60·5% of methicillin-resistant CoNS. Nevertheless, in the remaining isolates those authors had observed atypical PCR profiles. In accordance with these findings, our results suggest that the sequences analysed in the current study are not strictly associated with particular SCC*mec* types which could be in agreement with the fact that SCC*mec* are under continuous change [36]. Therefore, this typing strategy could be a useful epidemiological tool in the discrimination of methicillin-resistant staphylococci even though it may not be suitable for SCC*mec* type determination.

In conclusion, the current study shows that transmission of *S. epidermidis* between farmers and cattle may occur. Biofilm production was more typical for human than non-human isolates. MRSE isolates, in which a high variability of SCC*mec* was observed, were widespread in humans and cattle. Dairy products were not shown to be an important source of either human infections or methicillin-resistant strains of *S. epidermidis*.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Piette A, Verschraegen G.** Role of coagulase-negative staphylococci in human disease. *Veterinary Microbiology* 2009; **134**: 45–54.

2. **Gill SR, *et al.*** Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. *Journal of Bacteriology* 2005; **187**: 2426–2438.
3. **Vuong C, Otto M.** *Staphylococcus epidermidis* infections. *Microbes and Infection* 2002; **4**: 481–489.
4. **Taponen S, Pyorala S.** Coagulase-negative staphylococci as cause of bovine mastitis – not so different from *Staphylococcus aureus*? *Veterinary Microbiology* 2009; **134**: 29–36.
5. **Vadyvaloo V, Otto M.** Molecular genetics of *Staphylococcus epidermidis* biofilms on indwelling medical devices. *International Journal of Artificial Organs* 2005; **28**: 1069–1078.
6. **Sharma M, Anand SK.** Characterization of constitutive microflora of biofilms in dairy processing lines. *Food Microbiology* 2002; **19**: 627–636.
7. **Moretro T, *et al.*** Biofilm formation and the presence of the intercellular adhesion locus *ica*, among staphylococci from food and food processing environments. *Applied and Environmental Microbiology* 2003; **69**: 5648–5655.
8. **Becker K, *et al.*** Understanding the physiology and adaptation of staphylococci: a post-genomic approach. *International Journal of Medical Microbiology* 2007; **297**: 483–501.
9. **Ben Saida N, *et al.*** Clonality of clinical methicillin-resistant *Staphylococcus epidermidis* isolates in a neonatal intensive care unit. *Pathologie Biologie* 2006; **54**: 337–342.
10. **Sawant AA, Gillespie BE, Oliver SP.** Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk. *Veterinary Microbiology* 2009; **134**: 73–81.
11. **de Araujo GL, *et al.*** Commensal isolates of methicillin-resistant *Staphylococcus epidermidis* are also well equipped to produce biofilm on polystyrene surfaces. *Journal of Antimicrobial Chemotherapy* 2006; **57**: 855–864.
12. **Martineau F, *et al.*** Species-specific and ubiquitous DNA-based assays for rapid identification of *Staphylococcus epidermidis*. *Journal of Clinical Microbiology* 1996; **34**: 2888–2893.
13. **Frebourg NB, *et al.*** PCR-based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *Journal of Clinical Microbiology* 2000; **38**: 877–880.
14. **Schlegelova J, *et al.*** The biofilm-positive *Staphylococcus epidermidis* isolates in raw materials, foodstuffs and on contact surfaces in processing plants. *Folia Microbiologica* 2008; **53**: 500–504.
15. **Linhardt F, *et al.*** Pulsed-field gel electrophoresis of genomic restriction fragments as a tool for the epidemiologic analysis of *Staphylococcus aureus* and coagulase-negative staphylococci. *FEMS Microbiology Letters* 1992; **95**: 181–186.
16. **Pantucek R, *et al.*** Genomic variability of *Staphylococcus aureus* and the other coagulase-positive *Staphylococcus* species estimated by macrorestriction analysis using pulsed-field gel electrophoresis. *International Journal of Systematic Bacteriology* 1996; **46**: 216–222.
17. **Cucarella C, *et al.*** Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *Journal of Bacteriology* 2001; **183**: 2888–2896.
18. **Arciola CR, Baldassarri L, Montanaro L.** Presence of *ica*A and *ica*D genes and slime production in a collection of staphylococcal strains from catheter-associated infections. *Journal of Clinical Microbiology* 2001; **39**: 2151–2156.
19. **Oliveira DC, de Lencastre H.** Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 2002; **46**: 2155–2161.
20. **Oliveira DC, Milheirico C, de Lencastre H.** Redefining a structural variant of staphylococcal cassette chromosome *mec*, SCC*mec* type VI. *Antimicrobial Agents and Chemotherapy* 2006; **50**: 3457–3459.
21. **Miragaia M, *et al.*** Comparison of molecular typing methods for characterization of *Staphylococcus epidermidis*: proposal for clone definition. *Journal of Clinical Microbiology* 2008; **46**: 118–129.
22. **Milheirico C, Oliveira DC, de Lencastre H.** Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 2007; **51**: 3374–3377.
23. **Thorberg BM, *et al.*** Pheno- and genotyping of *Staphylococcus epidermidis* isolated from bovine milk and human skin. *Veterinary Microbiology* 2006; **115**: 163–172.
24. **Gillespie BE, *et al.*** Prevalence and persistence of coagulase-negative *Staphylococcus* species in three dairy research herds. *Veterinary Microbiology* 2009; **134**: 65–72.
25. **Meeniken D, *et al.*** Occurrence of MRSA in pigs and in humans involved in pig production – preliminary results of a study in the Northwest of Germany. *Deutsche Tierarztliche Wochenschrift* 2008; **115**: 132–139.
26. **Haertl R, Bandlow G.** Genotyping of *Staphylococcus epidermidis* by small-fragment restriction endonuclease analysis and pulsed-field gel electrophoresis of genomic restriction fragments. *Microbiology and Immunology* 1994; **38**: 527–534.
27. **Tondo EC, *et al.*** Assessing and analysing contamination of a dairy products processing plant by *Staphylococcus aureus* using antibiotic resistance and PFGE. *Canadian Journal of Microbiology* 2000; **46**: 1108–1114.
28. **Boynukara B, *et al.*** Evolution of slime production by coagulase-negative staphylococci and enterotoxigenic characteristics of *Staphylococcus aureus* strains isolated from various human clinical specimens. *Journal of Medical Microbiology* 2007; **56**: 1296–1300.
29. **Presterl E, *et al.*** Clinical behavior of implant infections due to *Staphylococcus epidermidis*. *International Journal of Artificial Organs* 2005; **28**: 1110–1118.
30. **Sanderson AR, Leid JG, Hunsaker D.** Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. *Laryngoscope* 2006; **116**: 1121–1126.

31. **Miyamoto H, *et al.*** Survey of nasal colonization by, and assessment of a novel multiplex PCR method for detection of biofilm-forming methicillin-resistant staphylococci in healthy medical students. *Journal of Hospital Infection* 2003; **53**: 215–223.
32. **Jimenez E, *et al.*** *Staphylococcus epidermidis*: a differential trait of the fecal microbiota of breast-fed infants. *BMC Microbiology* 2008; **8**: 143.
33. **Michelim L, *et al.*** Pathogenicity factors and antimicrobial resistance of *Staphylococcus epidermidis* associated with nosocomial infections occurring in intensive care units. *Brazilian Journal of Microbiology* 2005; **36**: 17–23.
34. **Abbassi MS, *et al.*** Clonality and occurrence of genes encoding antibiotic resistance and biofilm in methicillin-resistant *Staphylococcus epidermidis* strains isolated from catheters and bacteremia in neutropenic patients. *Current Microbiology* 2008; **57**: 442–448.
35. **Silva FR, *et al.*** Isolation and molecular characterization of methicillin-resistant coagulase-negative staphylococci from nasal flora of healthy humans at three community institutions in Rio de Janeiro City. *Epidemiology and Infection* 2001; **127**: 57–62.
36. **Hanssen AM, Sollid JUE.** SCC*mec* in staphylococci: genes on the move. *FEMS Immunology and Medical Microbiology* 2006; **46**: 8–20.
37. **Nunes SF, *et al.*** Technical note: antimicrobial susceptibility of Portuguese isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* in subclinical bovine mastitis. *Journal of Dairy Science* 2007; **90**: 3242–3246.
38. **Montanaro L, *et al.*** Antibiotic multiresistance strictly associated with IS*256* and *ica* genes in *Staphylococcus epidermidis* strains from implant orthopedic infections. *Journal of Biomedical Materials Research Part A* 2007; **83A**: 813–818.
39. **Machado ABP, *et al.*** Distribution of staphylococcal cassette chromosome *mec* (SCCmec) types I, II, III and IV in coagulase-negative staphylococci from patients attending a tertiary hospital in southern Brazil. *Journal of Medical Microbiology* 2007; **56**: 1328–1333.