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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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 mastitis 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 1a). 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

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

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).
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 1b) 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 (bioMe´rieux, 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 SmaI (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.
Identification and characterization of MRSE isolates

Isolates with MICs $\geq 0.5 \mu g/ml$ for OXA were tested for the presence of the mecA gene by PCR using MECA-P4 and MECA-P7 primers [19], HotStarTaq Master Mix kit (Qiagen, Germany) under the following conditions: initial denaturation at $94^\circ C$ for 15 min. followed by 30 cycles of denaturation at $94^\circ C$ for 1 min. annealing at $55^\circ C$ for 1 min. and extension at $72^\circ C$ for 1 min. with final extension at $72^\circ C$ for 5 min.

S. aureus ATCC 33591 and S. epidermidis ATCC 12228 strains served as positive and negative controls, respectively. In the mecA-positive isolates, the staphylococcal chromosomal cassette carrying the mecA gene (SCCmec) 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 ml PCR mixture and the final mixture contained the following: 0.5 $\mu M$ concentrations of each primer, 217 $\mu M$ each dNTP (Invitek, Germany), 2.5 $M$ MgCl2, 1 $r$ DNAzyme II PCR buffer and 0.8 $U$ DNAzyme 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 $94^\circ C$ for 5 min. followed by 35 cycles of denaturation at $94^\circ C$ for 30 s. annealing at $56^\circ C$ for 1 min. and extension at $72^\circ C$ for 1-5 min with final extension at $72^\circ C$ for 5 min.

S. aureus strains NCTC 10442 (SCCmec type I), N315 (SCCmec type II), 85/2087 (SCCmec type III), JCSC 4744 (SCCmec type IV) and WIS (SCCmec type V), kindly supplied by Teruyo Ito (Juntendo University, Tokyo, Japan), and S. aureus strain HDE288 (SCCmec 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 $\geq 79\%$. We also observed 13 clumps of isolates (not included in the clusters) in which only one of the PFGE types was found.
In Fig. 1. Ten (33.3%) isolates from human patients, a majority of the isolates (87; 71.9%) are indicated with respect to their origin, five groups (A–E) comprising isolates taken from the dairy plant. In an attempt to illustrate the genetic relatedness of the isolates with numbers of isolates (mostly 2–3), except for the 16 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 \textit{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 \textit{ica}-specific PCR (\textit{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 \textit{ica}-PCR, and three isolates were positive only by \textit{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 1b). 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 \textit{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...
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 SCCmec typing

Forty-five (37.2%) isolates with MICs ≥0.5 μg/ml for OXA were positive for mecA 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

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

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

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 mecA 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

<table>
<thead>
<tr>
<th>Origin of isolates</th>
<th>No.</th>
<th>PEN</th>
<th>OXA</th>
<th>CMP</th>
<th>TET</th>
<th>COT</th>
<th>ERY</th>
<th>CLI</th>
<th>CIP</th>
<th>GEN</th>
<th>TEI</th>
<th>VAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized patients</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Outpatients</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>3</td>
<td>0</td>
<td>10</td>
<td>12</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Farmers</td>
<td>14</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>22</td>
<td>21</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Final milk products</td>
<td>47</td>
<td>20</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Surface scrapings</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>76</td>
<td>45</td>
<td>6</td>
<td>23</td>
<td>28</td>
<td>64</td>
<td>13</td>
<td>19</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total, %</td>
<td>100</td>
<td>62.81</td>
<td>37.19</td>
<td>4.96</td>
<td>19.01</td>
<td>23.14</td>
<td>52.89</td>
<td>10.74</td>
<td>15.70</td>
<td>10.74</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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.
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\textit{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\textit{mec} types I–VI. The occurrence of two or more sequences, each specific for a different SCC\textit{mec} type, in a single isolate was observed quite often. A similar finding was also observed in four \textit{S. aureus} control strains. The PCR products from one selected isolate (SEP1692), which was positive for CIF, DCS, RIF, \textit{ccrB} and \textit{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\textit{mec} (12 different PCR patterns) and because no substantial correlation between particular sequences was observed, we determined the SCC\textit{mec} profiles using a numeric coding system as shown in Table 5. The prevalence of different SCC\textit{mec} profiles (Table 6a) and the PCR patterns detected in the control strains (Table 6b) are also shown. While a high variability of SCC\textit{mec} was found in the isolates of human origin, all of the MRSE isolates from dairy cattle had a uniform SCC\textit{mec} profile. In general, no substantial association between particular SCC\textit{mec} and PFGE types was observed. The same PFGE type was found in 9/18 MRSE isolates of SCC\textit{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 \textit{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 \textit{S. aureus} between humans and animals, very little is known about whether such a transmission may occur with \textit{S. epidermidis}. Thorberg et al. \([23]\), analysed \textit{S. epidermidis} from cows' milk and farmers' skin, and demonstrated that bovine \textit{S. epidermidis} mastitis may emanate from the farmers. In our study, two PFGE types which were identified in two isolates from farmers and two isolates from cattle both sharing the PFGE and SCC\textit{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 \textit{S. epidermidis}. Due to the fact that \textit{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 \textit{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 \textit{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

<table>
<thead>
<tr>
<th>First digit of numeric code</th>
<th>Second digit of numeric code</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIF</td>
<td>KDP</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

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, \textit{ccrB} and \textit{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.
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 1b). 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

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

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

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

<table>
<thead>
<tr>
<th>Source</th>
<th>SCC<em>mec</em> profile (numeric codes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 10* 11† 13 41 43 45 46 51 54 55 58</td>
</tr>
<tr>
<td>Hospitalized patients</td>
<td>— 1 — — 1 3 1 1 — 3 —</td>
</tr>
<tr>
<td>Outpatients</td>
<td>— 1 2 1 — 1 1 — 3 1 3 1</td>
</tr>
<tr>
<td>Farmers</td>
<td>1 — 1 — 1 1 — 2 — —</td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>— — — — 16 — — — —</td>
</tr>
<tr>
<td>Total</td>
<td>1 2 3 1 18 1 4 1 6 1 6 1</td>
</tr>
</tbody>
</table>

* 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).
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 meticillin 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 SCCmec profile as one of the isolates from an outpatient that had caused a post-operative 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 mecA gene and biofilm positivity was observed. The mecA gene was found in 13/21 biofilm-positive isolates. Moreover, mecA was also detected in 32 biofilm-negative isolates. However, almost all of the human biofilm-positive isolates (13/16) were also mecA-positive (only one biofilm-positive isolate from a hospitalized patient and two from farmers were mecA-negative). This indicates a high association between biofilm production and multi-resistance (meticillin 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 meticillin resistance was not observed in the current study in the non-human biofilm-forming isolates, since all of them (n = 5) were mecA-negative. Nevertheless, a high correlation between the presence of ica genes and meticillin resistance has already been determined in S. epidermidis from bovine milk [10].

Besides the high variability of SCCmec, the majority (88.9%) of the MRSE isolates could not be assigned to any of the known SCCmec types. This might be explained by the fact that the SCCmec 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 SCCmec in 60.5% of meticillin-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 SCCmec types which could be in agreement with the fact that SCCmec are under continuous change [36]. Therefore, this typing strategy could be a useful epidemiological tool in the discrimination of meticillin-resistant staphylococci even though it may not be suitable for SCCmec 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 SCCmec was observed, were widespread in humans and cattle. Dairy products were not shown to be an important source of either human infections or meticillin-resistant strains of S. epidermidis.

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DECLARATION OF INTEREST

None.

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