Methicillin-resistant \textit{Staphylococcus aureus} in cattle food chains –
Prevalence, diversity, and antimicrobial resistance in Germany\textsuperscript{1}

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\textbf{ABSTRACT:} Livestock-associated methicillin-resistant \textit{Staphylococcus aureus} (MRSA) have been found in various farm animal species throughout the world. It was the objective of this study to estimate the prevalence of MRSA in different cattle food chains (milk, beef, and veal) in Germany, to analyze the MRSA diversity along each food chain and to compare the characteristics of the different subtypes. Samples were collected between 2009 and 2012 from dairy herds (bulk tank milk), veal herds (dust from the stables), veal calves, and beef cattle at slaughter (nasal swabs) and carcasses of veal calves (surface cuts) and beef as well as veal at retail. Sampling was proportionally distributed over the country according to the cattle population (on-farm sampling), slaughterhouse capacity (abattoir samples), and the human population (meat at retail). Methicillin-resistant \textit{S. aureus} were isolated using harmonized methods from all sample types and populations investigated. The highest proportion of positive samples was found in nasal swabs from veal calves at slaughter in 2012 (144/320; 45.0%) and the lowest rate in bulk tank milk in 2009 (14/388; 4.1%). Most isolates, irrespective of the origin, were from spa types t011 and t034. Both have been assigned to the clonal complex (CC) 398. Few isolates (15/632; 2.4%) were from spa types not associated with the CC398. Spa-type patterns were similar along individual food chains but differed between food chains. Antimicrobial resistance patterns differed between isolates from the different food chains and spa types. Isolates from the veal chain displayed the highest resistance rates. We conclude that there is substantial diversity in the MRSA prevalence across different cattle production sectors.

\textbf{Key words:} antimicrobial resistance, cattle, food chain, methicillin, \textit{Staphylococcus aureus}

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\textbf{INTRODUCTION}

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) have been frequently detected in livestock in recent years. In cattle, first reports on MRSA date back to the 1970s, describing individual isolates from cases of mastitis in dairy cows (Devriese et al., 1972). In 2007, a report on transmission of MRSA in dairy cows and milking personal alerted people working with dairy cattle of the occupational health risks (Kaszanyitzky et al., 2007). In the following years a number of reports have described the prevalence of MRSA in dairy cattle and transmission of MRSA between people working on farms and dairy cattle (Antoci et al., 2013; Friedrich et al., 2011; Haran et al., 2012; Lim et al., 2013; Spohr et al., 2011). Likewise, calves on dairy farms were found positive for MRSA (Spohr et al., 2011). Only 1 study, testing a very small milk sample per herd, failed to detect MRSA (Virgin et al., 2009). A comparatively high prevalence of MRSA was found in veal calves (Graveland et al., 2012), while beef cattle in feedlots were tested negative in Canada (Weese et al., 2012). \textit{Staphylococcus aureus} is one of the leading causes of foodborne outbreaks due to its ability to produce staphylococcal enterotoxins (Hennekinne et al., 2012). Recently, a study conducted in Canada did not find any MRSA among \textit{S. aureus} isolates involved in staphylococcal food poisoning outbreaks (Crago et al., 2012). This is in line with the absence of enterotoxin genes.

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in MRSA from bulk tank milk or bovine meat (Argudin et al., 2011; Kreauusukon et al., 2012). So far, livestock-associated MRSA (LA-MRSA) are not considered to be transmitted via the ingestion of food.

This report describes the results of the investigations into MRSA in cattle from farm to fork, including dairy cattle, veal calves, beef animals, and food thereof. Our hypotheses were that 1) MRSA prevalence differs between production systems, 2) MRSA from dairy herds and veal calves are similar, as veal calves are frequently born in dairy herds, and 3) MRSA in meat mainly originate from primary production.

**MATERIALS AND METHODS**

**Sampling**

In Germany, a monitoring system for zoonotic bacteria in the food chain was established in 2009 to fulfill the requirements of directive 2003/99/EC (EU, 2003). The general aim of the monitoring system is to investigate the prevalence of zoonotic bacteria along the different food chains and to collect isolates of the different bacterial classes for further characterization, for example, typing and antimicrobial resistance (AMR) testing.

Sampling plans were designed to cover primary production in dairy cattle, beef cattle, veal calves, and meat at retail. Milk at retail was not included in the studies as milk is heat treated before being sold to the consumer with very few exceptions. Therefore, transmission of MRSA to milk at retail was not investigated. The exceptions were covered by a study on MRSA in bulk tank milk from dairy herds certified for marketing of raw milk to consumers. Within the monitoring system sampling plans are designed annually for collecting samples at farm, at the abattoir, and from food at retail. Sampling plans in the German system are negotiated between the federal institutions and the regional authorities to assure a high degree of compliance with the decided sampling procedures. This procedure has been fixed in national legislation (BMELV, 2012). Sampling at farm was distributed across the federal states proportionally to the number of animals kept. Sampling frequency at the abattoir was guided by the annual throughput of the abattoirs with respect to the animal category tested. Sampling at retail was proportional to the human population of the federal state as the focus here was on exposure of humans to MRSA via meat. Sample size was estimated as previously reported (Käsbohrer et al., 2010) based on an estimated prevalence of 50% as prior knowledge was not fully available. The numbers of samples taken per category are given in Table 1.

On dairy farms, bulk tank milk samples were collected (1 sample per farm per year) as MRSA, like other S. aureus, is a potent mastitis pathogen (Spohr et al., 2011). Dairy farms included randomly chosen conventional dairy farms with at least 20 lactating cows. In 2010, 30 so-called certified farms were additionally included. These farms are allowed to sell raw milk to consumers with few exceptions. Therefore, transmission of MRSA to milk at retail was not investigated. The exceptions were covered by a study on MRSA in bulk tank milk from dairy herds certified for marketing of raw milk to consumers. Within the monitoring system sampling plans are designed annually for collecting samples at farm, at the abattoir, and from food at retail. Sampling plans in the German system are negotiated between the federal institutions and the regional authorities to assure a high degree of compliance with the decided sampling procedures. This procedure has been fixed in national legislation (BMELV, 2012). Sampling at farm was distributed across the federal states proportionally to the number of animals kept. Sampling frequency at the abattoir was guided by the annual throughput of the abattoirs with respect to the animal category tested. Sampling at retail was proportional to the human population of the federal state as the focus here was on exposure of humans to MRSA via meat. Sample size was estimated as previously reported (Käsbohrer et al., 2010) based on an estimated prevalence of 50% as prior knowledge was not fully available. The numbers of samples taken per category are given in Table 1.

<table>
<thead>
<tr>
<th>Food chain</th>
<th>Sample type</th>
<th>Unit</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy cattle</td>
<td>Bulk tank milk; conventional farms</td>
<td>No.²</td>
<td>14/338</td>
<td>4.1 (2.0–6.3)</td>
<td>4.7 (2.3–7.1)</td>
<td>14/297</td>
</tr>
<tr>
<td></td>
<td>Bulk tank milk; certified farms³</td>
<td>No.²</td>
<td>3/30</td>
<td>10.0 (0–20.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veal calves⁴</td>
<td>Dust samples⁵</td>
<td>No.²</td>
<td>58/296</td>
<td>19.6 (15.1–24.1)</td>
<td></td>
<td>46/240</td>
</tr>
<tr>
<td></td>
<td>Nasal swabs at slaughter</td>
<td>No.²</td>
<td>123/350</td>
<td>35.1 (30.1–40.1)</td>
<td>144/320</td>
<td>45.0 (39.6–50.5)</td>
</tr>
<tr>
<td></td>
<td>Carcass at slaughter</td>
<td>No.²</td>
<td></td>
<td>% (95% CI)</td>
<td>96/312</td>
<td>30.8 (25.9–36.1)</td>
</tr>
<tr>
<td></td>
<td>Veal at retail</td>
<td>No.²</td>
<td>48/387</td>
<td>12.4 (9.1–15.7)</td>
<td>44/421</td>
<td>10.5 (7.9–13.8)</td>
</tr>
<tr>
<td></td>
<td>Meat preparations with veal</td>
<td>No.²</td>
<td>6/31</td>
<td>19.4 (5.4–33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef animals</td>
<td>Nasal swabs at slaughter</td>
<td>No.²</td>
<td></td>
<td></td>
<td>25/288</td>
<td>8.7 (5.9–12.5)</td>
</tr>
<tr>
<td></td>
<td>Beef at retail</td>
<td>No.²</td>
<td></td>
<td></td>
<td>41/509</td>
<td>8.1 (6.0–10.8)</td>
</tr>
</tbody>
</table>

¹Data have been published in annual reports (BVL, 2011, 2012, 2013, 2014).
²Number of positive samples/no. of samples.
³Farms producing certified milk (Vorzugsmilch) according to German law (Anonymous, 2007).
⁴In 2009 and 2010, cattle up to the age of 8 mo were included, and in 2012 cattle, up to the age of 12 mo were included.
⁵Numbers refer to (positive) pools of samples.
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\textit{Vorzugsmilch} but have to take additional hygienic measures in comparison to conventional farms (Bundesrepublik Deutschland, 2007). Regional authorities were advised to collect samples from all the farms of this type in their region.

On veal calf farms, 5 dust samples were collected from different surfaces of the stable and were pooled for analysis (Wagenaar and van de Giessen, 2009). Veal calves are typically raised to the age of 8 mo mostly on liquid feed (milk or milk replacer) to produce veal. In 2012, veal calf herds and farms housing animals up to 12 mo were included to be in line with recent recommendations from the European Food Safety Authority (EFSA, 2012).

At the abattoir, nasal swabs were collected from 1 animal per slaughter batch and excision samples (1 per slaughter batch) were collected from carcasses of veal calves and young cattle up to the age of 12 mo. In 2011, beef animals were sampled. Those are typically 18 to 30 mo old at slaughter and may have been raised under intensive conditions (confined housing for the complete lifetime) or under semi-intensive conditions (free range housing with suckler cows up to weaning and confined housing thereafter).

At retail, beef, veal, and meat preparations from veal were sampled. Food items covered by the inclusion criteria were sampled. Sampling personnel made sure that only 1 sample per production batch was collected.

Samples were collected by veterinary officials of the federal states and transported to the laboratory in cooled containers with the exception of dust samples that did not have to be cooled. Methicillin-resistant \textit{S. aureus} were isolated from the samples by the regional laboratories of the individual federal states according to presribed methods.

\textbf{Isolation of Methicillin-Resistant Staphylococcus aureus}

Regional laboratories were provided with a standard method recommendation for the isolation of MRSA by the National Reference Laboratory (NRL: Berlin, Germany) for coagulase positive staphylococci including \textit{S. aureus} (NRL-Staph) at the Federal Institute for Risk Assessment (Berlin, Germany). The 5 dust samples were pooled per farm in 100 mL Mueller Hinton broth supplemented with 6.5\% (6.0\%) NaCl for pre-enrichment (MHB+). Milk samples (25 mL), fresh meat (25 g), and meat preparations (25 g) were pre-enriched in 225 mL MHB+. After incubation for 16 to 20 h at 37°C, 1 mL pre-enrichment broth was transferred into 9 mL of tryptic soy broth supplemented with 3.5 mg/L cefoxitin and 75.0 (50.0) mg/L aztreonam, respectively. In January 2011, the 2 enrichment broths were slightly modified following an internal evaluation process (unpublished data). Salt content of Mueller Hinton broth was slightly reduced (from 6.5 to 6.0\%). Likewise, the aztreonam content of the tryptic soy broth was reduced from 75 to 50 mg/L. After incubation of this selective-enrichment broth for a further 16 to 20 h at 37°C, 1 loopful was plated onto chromogenic MRSA screening agar and incubated for 24 to 48 h at 37°C. Presumptive MRSA isolates were sent to the NRL-Staph for confirmation, typing, and further analysis.

\textbf{Confirmation and Molecular Typing}

Presumptive MRSA isolates were confirmed by an in-house multiplex PCR simultaneously targeting the 23S rDNA specific for \textit{Staphylococcus} species, the nuclease gene \textit{nucc}, which is specific for \textit{S. aureus}, and the resistance gene \textit{mecz} (Poulsen et al., 2003). Template DNA was extracted from isolates using commercial kits (RTP Bacteria DNA Mini Kit; Invitek, Berlin, Germany; and DNeasy Blood and Tissue kit; Qiagen, Hilden, Germany). All MRSA isolates were further characterized using \textit{spa} typing (Shopsin et al., 1999) and staphylococcal cassette chromosome \textit{mec} typing (Zhang et al., 2005), the latter differentiating between \textit{SCCmec} types I to V, including \textit{V}* (Argudin et al., 2010). The software Ridom Staphytype (Ridom GmbH, Würzburg, Germany) was used to assign \textit{spa} types. \textit{Spa} types that had not been identified and assigned to a clonal complex (CC) by the NRL before were additionally subjected to multilocus sequence typing (Enright et al., 2000).

\textbf{Antimicrobial Susceptibility Testing}

All isolates were tested for the susceptibility to antimicrobials using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). Commercial microtiter plates were used (TREK Diagnostic Systems, Magellan Biosciences, West Sussex, England). Evaluation of the minimum inhibitory concentrations was based on epidemiological cut-off values (ECOFF) published by the \textit{European Committee on Antimicrobial Susceptibility Testing} for MRSA and/or \textit{S. aureus} (EUCAST, 2014). Minimum inhibitory concentration values above the ECOFF indicated microbiological resistance. Minimum inhibitory concentration values lower or equal to the ECOFF characterized susceptible strains. \textit{Staphylococcus aureus} strain ATCC 25923 was used for quality assurance. The following antimicrobials were tested (ECOFF [mg/L] are shown in brackets): gentamicin ($\leq$2), kanamycin ($\leq$8), chloramphenicol ($\leq$16), ciprofloxacin ($\leq$1), tetracycline ($\leq$1), clindamycin ($\leq$0.25), erythromycin ($\leq$1), mupirocin ($\leq$1), linezolid ($\leq$4), vancomycin ($\leq$2), and the combination of quinupristin and dalfopristin ($\leq$1).
Statistical Analysis

Statistical analyses were performed using PASW (Predictive Analytics Software) Statistics (version 18.02; IBM Deutschland, Ehningen, Germany) and the open source software R (R Core Team, 2012). Prevalence estimates of MRSA were compared by simple chi-square test where appropriate. The number of MRSA isolates included in further analyses is not exactly congruent to the number of positive samples obtained within the national monitoring. The NRL did not always receive the corresponding isolate from the regional laboratories. Moreover, isolates that did not exactly correspond to the monitoring sampling plan but were from the target population were excluded from prevalence estimations but included in further typing and resistance testing. Although not all isolates were available for confirmation at the NRL-Staph, all samples reported positive by the regional laboratories were considered positive for the prevalence estimates. Prevalence of MRSA was only compared if the same kind of samples was collected at the same stage of the food chain.

Spa types were categorized in 4 different categories. Types t011 and t034 were 2 separate categories, as they were identified in most isolates. Other spa types that have been assigned to CC398 were categorized together as “other CC398.” The fourth category consisted of those isolates that were not assigned to CC398 and named non-CC398.

Antimicrobial resistance in MRSA was analyzed using logistic regression by substance. Only substances showing differences in resistance rates of more than 20% between isolates of different sources or isolates of different spa types were included in the testing. In the logistic regression model the outcome considered was resistant (1) or nonresistant (0). Food chain (dairy vs. beef vs. veal), spa type group, and SCCmec type were included as categorical covariates.

The degree of similarity between the frequency distributions of spa types of MRSA among the sample sets from the cattle food chains was estimated using the Czekanowski index or proportional similarity index (PSI; Rosef et al., 1985). It is calculated by

\[
PS = 1 - 0.5 \sum_{i} |p_i - q_i| = \sum_{i} \min(p_i,q_i),
\]

in which \(p_i\) and \(q_i\) represent the proportion of strains out of all strains among the data sets P and Q, which agree in the realization \(i\) of the variable of interest. The values for proportional similarity (PS) range from 1 for identical frequency distributions of the variable of interest to 0 for no similarities between the data sets. Since the size of the samples is rather small, a realization of the PSI may deviate largely from its true value. Therefore, the PSI was bootstrapped, obtaining a probability density distribution from which we derived the 95% confidence interval (95% CI) for the PSI. The statistic open source software R (R Core Team, 2012) was used to calculate the approximate confidence interval of the Czekanowski index using the nonparametric bootstrap BC\(_{0.95}\) method using 2,000 iterations (Efron and Tibshirani, 1986).

RESULTS

Prevalence

Methicillin-resistant \textit{S. aureus} were detected in all types of samples taken (Table 1). Prevalence was highest in veal calves. Herd level prevalence in 2010 and 2012 was identical and the prevalence in nasal swabs at the abattoir increased from 2009 to 2012. Prevalence in nasal swabs from beef cattle at slaughter was substantially lower. In dairy cows, herd level prevalence was similar in both years (2009 and 2010). It was numerically higher in the samples from certified farms, but the number of samples was low and therefore the difference was not significant.

Typing Results

A total of 632 isolates were confirmed as MRSA at the NRL-Staph (Table 2). Overall, 28 different spa types were identified among these isolates. Spa types t011 (58.1%) and t034 (32.0%), both assignable to CC398, predominated with a combined proportion of 90.0% of all isolates tested, ranging from 83.3 to 96.6% per sample type and year. Other spa types were also mostly assignable to the CC398 (7.6%; range 0 to 16.7%). Non-CC398 spa types were rare (15 isolates, 2.4%) and mostly identified in retail meat (12/15 isolates; 8.3% of the 142 isolates from meat). Only 3 of the 490 isolates that did not originate from retail meat were non-CC398 (0.6%). Those were identified as t002, t009, and t1919 and were isolated from herds of veal calves at farm (2 isolates) or veal calves at slaughter (1 isolate).

Diversity of MRSA tended to be minimal in bulk milk tank samples that harbored only 3 different spa types (29 isolates). In contrast, 11 different spa types were isolated from dust samples from veal farms, veal calves at slaughter, and veal at retail (Table 2).

Three different specific SCCmec types were identified. Staphylococcal cassette chromosome \textit{mec V} was the most frequent type with 75.3% of the isolates (Fig. 1). Type IVa was the second most frequent (18.5%) and type V* the least frequent (3.0%). Some isolates were not typeable (3.0%).

Staphylococcal cassette chromosome \textit{mec V} type V was most frequent in all spa types assigned to CC398 (Fig. 1). Type IVa occurred frequently in t011 and other CC398 but was rare in t034. It was the most frequent type in the non-CC398 isolates. Staphylococcal cassette chromosome \textit{mec V} type V* was mainly observed in t034
isolates (19/20 type V* isolates) where it accounted for 9.6% of all isolates. Of the 20 isolates that were not typeable concerning their SCCmec type, 14 were spa type t011 and the others were non-CC398 isolates.

**Similarities between spa-Type Patterns at the Different Stages of the Food Chain**

Overall, spa-type patterns were similar within the same food chain (Table 2). Most of the isolates found in nasal swabs of veal calves at slaughter were from spa types that also had been isolated from dust on veal calf farms (251/261; 96.2%). Likewise, isolates found on carcasses mostly were from spa types that were also found in nasal swabs (97.8%). In veal at retail, 10% of the isolates were from spa types that had not been identified in carcass swabs. Moreover, 7 of these 10 isolates were from spa types that were not identified in any other sample from the veal food chain.

In beef, none of the non-CC398 associated spa types were identified in nasal swabs at slaughter. Figure 2 displays the quantification of the similarity of the spa type pat-
terns in the veal food chain using the PSI. The index was fairly high for all pairs analyzed. The index was highest between the isolates from nasal swabs from veal calves at slaughter and those from the carcasses sampled in the same year (0.89; 95% CI 0.79–0.97) and between dust samples on farm and nasal swabs at slaughter sampled in the same year (0.86; 95% CI 0.69–0.97). It was somewhat lower when isolates from meat at retail were compared with those from primary production or at slaughter. It was also lower for the comparison of isolates from 2 different sampling years, that is, 2009 and 2012.

Antimicrobial Resistance

Of the 632 isolates tested only 1 isolate was not resistant to any further antimicrobial than β-lactams. Antimicrobial resistance varied between the 3 food chains with the veal food chain showing the highest number of resistances in the isolates (median 5 substances vs. 3 in beef chain and 4 in dairy cattle; \( P < 0.01 \)).

Considering the individual substances, 5 of the 11 substances showed minimal variation between food chains and subtypes of MRSA because either nearly all isolates were susceptible (chloramphenicol, mupirocin, linezolid, and vancomycin) or most isolates were resistant (tetracycline; Table 3). Only some non-CC398 isolates were susceptible to tetracycline (4/15; 26.7%).

Further statistical analyses were restricted to the other 6 substances (Table 4). Significant differences in the resistance rates between the food chains were observed for gentamicin, kanamycin, erythromycin, and clindamycin. In all cases the odds of resistance to the respective substance were lower for isolates from the beef chain compared to those from the veal chain. No significant difference was observed between the resistance rates in isolates from dairy cattle chain and the other chains.

Spa types were associated with AMR to all the 6 substances (Fig. 3). Staphylococcal cassette chromosome mec type was associated to resistance against 5 of the 6 substances (all except ciprofloxacin). Interestingly, all significant associations indicated that SCCmec type V was less likely resistant than the other less frequent SCCmec types.

Three odds ratios were not calculated as either all or none of the isolates were resistant. None the 29 dairy cattle isolates was resistant to ciprofloxacin, while 13.6% of the isolates from the beef and the veal food chain were resistant to this fluoroquinolone (Table 3). Staphylococcal cassette chromosome mec type V* was consistently susceptible to gentamicin and ciprofloxacin.

DISCUSSION

This is the first description of results of representative studies on MRSA along several cattle food chains of a country. The results show that, in Germany, MRSA can be found in dairy, beef, and veal production systems including the meat and milk produced from animals raised in these systems. Concerning dairy cattle and veal calves the results confirm other studies that were published previously (Friedrich et al., 2011; Graveland et al., 2010; Spohr et al., 2011; Vanderhaeghen et al., 2010). Studies in beef

Table 3. Antimicrobial resistance (%) in methicillin-resistant *Staphylococcus aureus* isolates from different stages of different cattle food chains in 2009 through 2012

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Veal calf dust sample</th>
<th>Veal calf nasal swab</th>
<th>Veal calf carcass</th>
<th>Veal chain, total</th>
<th>Beef cattle nasal swab</th>
<th>Beef chain, total</th>
<th>Bulk tank milk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
<td>83</td>
<td>261</td>
<td>90</td>
<td>103</td>
<td>537</td>
<td>27</td>
<td>39</td>
<td>66</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>25.3</td>
<td>37.5</td>
<td>33.3</td>
<td>21.4</td>
<td>31.8</td>
<td>3.7</td>
<td>17.9</td>
<td>12.1</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>41.0</td>
<td>49.8</td>
<td>44.4</td>
<td>35.0</td>
<td>44.7</td>
<td>11.1</td>
<td>38.5</td>
<td>27.3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>75.9</td>
<td>69.3</td>
<td>70.0</td>
<td>60.2</td>
<td>68.7</td>
<td>29.6</td>
<td>64.1</td>
<td>50.0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>81.9</td>
<td>78.5</td>
<td>74.4</td>
<td>68.0</td>
<td>76.4</td>
<td>37.0</td>
<td>59.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>7.2</td>
<td>5.4</td>
<td>6.7</td>
<td>5.8</td>
<td>6.0</td>
<td>7.4</td>
<td>7.7</td>
<td>7.6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>99.0</td>
<td>99.8</td>
<td>100.0</td>
<td>92.3</td>
<td>95.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18.1</td>
<td>14.6</td>
<td>10.0</td>
<td>10.7</td>
<td>13.6</td>
<td>7.4</td>
<td>17.9</td>
<td>13.6</td>
</tr>
<tr>
<td>Synercid</td>
<td>48.2</td>
<td>44.4</td>
<td>46.7</td>
<td>37.9</td>
<td>44.1</td>
<td>29.6</td>
<td>35.9</td>
<td>33.3</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>2.0</td>
<td>0.0</td>
<td>1.0</td>
<td>3.0</td>
<td>0.0</td>
<td>4.0</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>2.0</td>
<td>0.0</td>
<td>1.0</td>
<td>3.0</td>
<td>3.0</td>
<td>0.0</td>
<td>5.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Figure 2. Proportional similarity index (PSI) and confidence intervals (error bars) for spa types of isolates from different years and from different stages of the veal food chain.
animals are rare so far. A Canadian study did not detect MRSA in feedlot cattle (Weese et al., 2012). Methicillin-resistant *S. aureus* in beef had been reported previously from the Netherlands and the United States, albeit at low proportions (de Boer et al., 2009; Jackson et al., 2013). At the same time, several studies failed to detect MRSA in beef (Buyukcangaz et al., 2013; Hanson et al., 2011).

**Prevalence and Typing Results**

Results for carcasses at slaughter as well as the similarity between *spa*-type patterns in the environment of the animals and their nasal swabs, their carcasses, and meat thereof indicate that MRSA are readily transmitted to the carcass during slaughter and also further down the food chain during processing. Veal calves mostly originate from dairy herds. Therefore, MRSA in the calves may originate from the dairy production system. This is in line with the results of our study, as 2 of the 3 *spa*

![Figure 3. Antimicrobial resistance in isolates from different *spa*-type categories (*n* = 632).](image)

Table 4. Association of antimicrobial resistance to selected substances, typing results and food chain (*n* = 632). Results of logistic regression for each substance including food chain, *spa* type, and staphylococcal cassette chromosome (SCC) *mec* type as covariates models. Significant associations are depicted in bold. “Veal,” “t011,” and “SCC-mec type V” were the reference categories for all analyses.

<table>
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<th>Food chain</th>
<th>OR&lt;sup&gt;1&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;–&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;+&lt;/sup&gt;</th>
<th><em>Spa</em> category&lt;sup&gt;4&lt;/sup&gt;</th>
<th>OR</th>
<th>CI&lt;sup&gt;–&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;+&lt;/sup&gt;</th>
<th>SCC&lt;sup&gt;5&lt;/sup&gt; <em>mec</em> type</th>
<th>OR</th>
<th>CI&lt;sup&gt;–&lt;/sup&gt;</th>
<th>CI&lt;sup&gt;+&lt;/sup&gt;</th>
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<td>0.75</td>
<td>t034</td>
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<td>0.00</td>
<td>0.07</td>
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<td>V&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2.20</td>
<td>0.58</td>
<td>8.35</td>
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<sup>1</sup>OR = Odds ratio.

<sup>2</sup>CI<sup>–</sup> = confidence interval -. 

<sup>3</sup>CI<sup>+</sup> = confidence interval +.

<sup>4</sup>CC = clonal complex.

<sup>5</sup>No OR calculated as all isolates were susceptible.

<sup>6</sup>n.t. = not typeable.
However, as it was infrequent in dairy herds it may have escaped detection in the veal or beef herds.

The prevalence of MRSA in dairy herds was based on bulk tank milk samples. Recently, an Italian study performed in herds that were suspected to be MRSA positive indicated that bulk tank milk samples may underestimate the prevalence of MRSA in dairy herds. However, the authors used a selective broth with much higher levels of antimicrobials and did not report the amount of milk included in the sample (Antoci et al., 2013). It is not clear whether this may have hampered sensitivity of bulk tank milk analysis.

The diversity of spa types was higher in veal calves than in dairy cattle. This has been explained by the diverse origin of the calves raised on veal farms. For the veal industry of the Netherlands it has been reported that the calves originated from a number of different European Union member states (Wagenaar and van de Giesen, 2009). In contrast to pigs or poultry, where sows and hens produce 25 piglets and more than 200 chicks per year, cows usually have 1 calf. Therefore, the number of calves born on a dairy farm is limited. Veal calf herds need to purchase animals from a great variety of farms or through markets or traders. On the one hand this increases the risk that at least 1 of the calves originates from a MRSA-positive dairy farm. On the other hand, these veal farms frequently use antimicrobials to counteract disease conditions associated with crowding; hence, MRSA introduced by individual positive calves are exposed to highly favorable conditions of selection pressure towards AMR (Niedersächsisches Ministerium für Ernährung Landwirtschaft Verbraucherschutz und Landesentwicklung and Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, 2011; Pardon et al., 2012).

The proportion of MRSA positive beef animals at slaughter was substantially lower than that observed for veal calves. Animals at slaughter do not exactly reflect the situation on farm as bacterial colonization may also have been acquired during transport or in the lairage facilities as reported for MRSA in pigs (Broens et al., 2011). However, transport and lairage are factors that all large slaughter animals are exposed to. Therefore, the difference observed in the prevalence at slaughter may indicate a similar difference in primary production, which is in line with the observed data on MRSA in veal calves at farm. A different level of antimicrobial use between beef andveal animals could have contributed to the differences. Differences in the level of antimicrobial use have recently been reported for Lower Saxony, the German federal state housing a substantial part of the German veal industry (Niedersächsisches Ministerium für Ernährung Landwirtschaft Verbraucherschutz und Landesentwicklung and Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, 2011).

The rate of positive veal carcasses was high as compared to data available for pigs (Beneke et al., 2011; Kastrup, 2011). The reason for this remains to be elucidated. In pigs, comparatively low detection rates on carcasses were explained by heat treatments applied to the carcass surfaces during the slaughter process (Lassok and Tenhagen, 2013). Such treatments are not applied to cattle. However, as the skin is removed a massive reduction of the contamination could have been expected and has been reported with respect to verotoxigenic Escherichia coli (VTEC; Thomas et al., 2012). It is not clear why, with respect to MRSA, no such reduction occurs. In contrast to VTEC, MRSA is not an enteric pathogen and therefore fecal recontamination is not a likely source of the isolates on the surface of carcasses. A potential role of contaminated slaughter equipment needs to be investigated as S. aureus is well known for its ability to form biofilms (Kusumaningrum et al., 2003). Moreover, aerosols associated with the mechanic removal of the skin could be involved in the contamination of carcasses (Schmidt et al., 2012). Slaughterhouse personnel may be involved in the transmission; however, they are not a likely source of MRSA as all of the MRSA on the carcasses were from the CC398 that is still infrequent in the human population and in slaughter personnel that do not handle live animals (Mulders et al., 2010; Van Cleef et al., 2010).

In retail meat, diversity of strains was high. Veal and beef at retail not necessarily is derived from domestic production and divergent strains may simply reflect a different origin. However, most of the isolates were from spa types that had also been observed in primary production and animals at slaughter, which supports the hypothesis that MRSA in meat from cattle mainly originate from primary production. The comparatively high proportion of non-CC398 strains in meat at retail (8.3%) and the lower PSI observed when comparing meat at retail with carcasses or animal samples suggests that additional clones of MRSA are transmitted to meat that probably do not originate from primary production but from people handling the meat during processing or at retail. Yet, compared to the CC398 strains, the proportion is comparatively small and primary production therefore can be considered the main source of MRSA on retail meat.

Traded slaughter animals may not be an explanation as MRSA in veal calves in the Netherlands are also mainly from CC398 (Graveland et al., 2010).

In contrast to the situation in turkey meat the non-CC398 MRSA found in beef and veal do not belong to 1 or 2 other distinct CC but are more diverse. In the turkey meat food chain it could be shown that most of the non-CC398 strains occurring in meat were from 2 distinct spa types, that is, t002 (CC5) and t1430 (CC9), that were also frequently found in primary production (Vossenkuhl et al., 2014).
The association of the 2 spa types t011 and t034 with certain SCCmec types has been reported before. In a study in slaughter pigs in Germany, most of the isolates harboring SCCmec type V* were from spa type t034 (94.0%; Tenhagen et al., 2009). The similarity of this pattern indicates that the same MRSA clones that spread in the pig population in Germany can also be found in the cattle population. However, in-depth molecular–biological analyses are needed to confirm this hypothesis.

**Antimicrobial Resistance**

Antimicrobial resistance was high in the MRSA isolates from all sample types but highest in veal calves. This adds to the observed higher frequency of MRSA in the veal calf chain. This is not surprising given the massive exposure of veal calves to antimicrobials (Pardon et al., 2012; Wagenaar and van de Giessen, 2009). Although intensively housed beef cattle are also frequently exposed to antimicrobials, exposure is substantially lower than in veal calves (Niedersächsisches Ministerium für Ernährung Landwirtschaft Verbraucherschutz und Landesentwicklung and Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, 2011).

As previously described for LA-MRSA from animal origin in Germany (Argudin et al., 2011; Tenhagen et al., 2009), resistance to tetracycline was common with only few isolates susceptible to this antimicrobial. Likewise, resistance to clindamycin and erythromycin was widespread. However, resistance to these antimicrobials was significantly higher in the veal chain than in the beef chain. The same applied for resistances to gentamicin.

Aminoglycosides, macrolides, and lincosamides are commonly used in veal calves but also in beef cattle (Niedersächsisches Ministerium für Ernährung Landwirtschaft Verbraucherschutz und Landesentwicklung and Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, 2011), although less frequently. Resistance of isolates from dairy cows was numerically lower than those from veal and beef cattle, but due to the low number of isolates from bulk tank milk the differences were not significant.

Antimicrobial resistance was also associated with spa types. This has been observed before (Tenhagen et al., 2009). The reasons for the differences in the resistance patterns of the different spa types are not clear. spa types t011 and t034 differ substantially with respect to AMR, although both spa types were frequent in all sample materials. Recently, t034 clustered separately in a study using whole genome sequencing (Price et al., 2012). This indicates that t034 is probably a distinct clone that differs substantially from t011, although the spa-repeat patterns are very similar. This adds to the difference observed with respect to the SCCmec types.

Resistance patterns differed between non-CC398 isolates and CC398 isolates. A lower resistance rate to tetracycline and a higher resistance rate to ciprofloxacin indicate that there might be human-associated strains among these isolates, as ciprofloxacin resistance is typical for hospital-acquired MRSA and resistance to tetracycline is infrequent in these isolates (Layer and Werner, 2013). Again, these findings call for in-depth molecular comparison of the strains.

**Conclusions**

Methicillin-resistant *S. aureus* prevalence differs between the 3 cattle production systems compared, with the veal chain displaying the highest prevalence. Most of the isolates from veal calves are from the same spa types observed in dairy herds; however, overall diversity seems to be higher in calves. Methicillin-resistant *S. aureus* in meat (veal and beef) are very similar to those for primary production indicating transmission of the bacteria along the food chain. However, data also indicate that further MRSA clones of potentially human origin may be introduced into the cattle food chains during processing.

**LITERATURE CITED**


Methicillin-resistant *Staphylococcus aureus* in cattle in Germany


