Review

From Pig to Pork: Methicillin-Resistant *Staphylococcus aureus* in the Pork Production Chain

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major global public health concern and could be a food safety issue. Recurrent reports have documented that pig herds are an important reservoir for MRSA, specifically the livestock-associated sequence type 398. The high prevalence of MRSA in pig primary production facilities and the frequent detection of MRSA of the same types in pork and pig meat products raise the question of underlying mechanisms behind the introduction and transmission of MRSA along the pork production chain. A comprehensive review of current literature on the worldwide presence of livestock-associated MRSA in various steps of the pork production chain revealed that the slaughter process plays a decisive role in MRSA transmission from farm to fork. Superficial heat treatments such as scalding and flaming during the slaughter process can significantly reduce the burden of MRSA on the carcasses. However, recontamination with MRSA might occur via surface treating machinery, as a result of fecal contamination at evisceration, or via increased human handling during meat processing. By optimizing processes for carcass decontamination and avoiding recontamination by effective cleaning and personal hygiene management, transmission of MRSA from pig to pork can be minimized.

*Staphylococcus aureus* is known as a frequent commensal and pathogen of humans and animals. It can persistently or intermittently colonize the skin and the mucous membranes of the upper respiratory, gastrointestinal, and lower urogenital tracts. Nasal carriage of the organism has been identified as the most important risk factor for the development of infections, resulting from skin and soft tissue injury (58). Problems associated with *S. aureus* infection include superficial skin disease, systemic disease, and toxicoses (47). In livestock, *S. aureus* is a major cause of mastitis in dairy cows and of various types of necroses in poultry flocks (40).

The ability of *S. aureus* to adapt to selective pressures has facilitated the development of antimicrobial resistance and particularly the spread of methicillin-resistant strains in health care institutions, the community, and livestock herds. Methicillin resistance results from the acquisition of the *mecA* gene, which codes for an alternative penicillin binding protein (PBP2’ or PBP2a). The modified surface protein has a low binding affinity to β-lactam antibiotics and thus their bactericidal effects are reduced. The *mecA* gene is chromosomally inserted as part of the mobile genetic element staphylococcal cassette chromosome (SCC) *mec*. Depending on the type of SCC*mec*, the added DNA can also carry antibiotic resistance genes on integrated plasmids, leading to multidrug resistance (31).

Since the detection of methicillin-resistant *S. aureus* (MRSA) in milk from mastitic cows in 1972, interest in animals as a reservoir for MRSA has increased (32). Several investigators have isolated MRSA from companion and livestock animal species (62). Whereas MRSA in companion animals is mainly associated with classical human strains, a distinct MRSA clone has emerged in livestock (21).

Livestock-associated (LA) MRSA strains are nontypeable with pulsed-field gel electrophoresis using the standard restriction endonuclease *Sma*I. Based on multilocus sequence typing, a method of defining MRSA strains by the allelic profile of seven housekeeping genes (35), sequence type (ST) 398 was identified as predominant in livestock (38, 56, 93). Related sequence types that share at least five identical sequenced housekeeping genes have been grouped within clonal complex (CC) 398 using the BURST algorithm (Based Upon Related Sequence Types, University of Bath, Bath, UK). The clonal complex was named after ST398, the ancestor strain with the largest number of single-locus variants in the group (35). Various *Staphylococcus* protein A gene (*spa*) types have been assigned to CC398, with t011, t034, and t108 the dominant types (38). *spa* types are defined by single locus DNA sequencing of the polymorphic region of the *spa* gene. The sequence and order of the repeats determine the *spa* type of the strain (41). LA-MRSA strains mainly carry SCC*mec* types IVa, V, and a variant of V, coding for resistance against tetracycline and frequent resistance against macrolides, lincosamides, aminoglycosides, trimethoprim, and fluoroquinolones. The common
absence of Panton-Valentine leukocidin (PVL) and various other virulence factors differentiates LA-MRSA from community-associated (CA) MRSA strains (4). Table 1 compares the main features of LA-MRSA, hospital-associated MRSA, and CA-MRSA strains.

Pig primary production was identified as one of the most important reservoirs for LA-MRSA. Retrospective analysis of preserved isolates indicated that the major clone has been present in the pig population in Germany at least since 2004 (69), which coincides with its first isolation from a pig and its farmer in The Netherlands in the same year (110).

Subsequently, pig primary production, including downstream industries, was the subject of numerous investigations to determine the respective LA-MRSA detection rate. The increasing number of reports of LA-MRSA in livestock-derived food products raises the question of how the organism spreads at different stages of the pork production chain.

This review includes current literature on the worldwide presence of LA-MRSA at various stages of the pork production chain with respect to prevalence and dominant lineages in different geographical regions. The scopus (http://www.scopus.com) and PubMed (http://www.pubmed.com) databases were searched using the keywords “MRSA” and “Staphylococcus aureus” in combination with “ST398,” “CC398,” “pig,” “meat,” “food,” “slaughter,” “hygiene,” or “hospital.” Sources listed in the available studies also were cross-checked.

The first part of this review compiles published investigations of LA-MRSA in the pig primary production chain, including an overview of analyzed risk factors for inter- and intraherd transmission. The second part includes recent findings relating to the incidence of LA-MRSA during slaughter and further meat processing and on the final meat product. These data are used to determine the critical processes for the transmission of MRSA in the pork production chain. The public health relevance of LA-MRSA at various different steps of the pork production chain also is discussed.

**MRSA PREVALENCE IN PIG PRIMARY PRODUCTION**

Since the first report of the presence of MRSA in the meat producing pig population and of a regional high carriage rate of MRSA among pig farmers in The Netherlands in 2005, an awareness of MRSA in livestock has increased (110). Several studies have been conducted in various countries to assess the prevalence of MRSA, to understand the dynamics of spread within the pig primary production sector, and to determine the public health relevance.

Within a comprehensive baseline study in 2008, the European Food Safety Authority (EFSA) (38) detected MRSA-positive breeding herds in 12 of 26 European countries. The MRSA prevalence among pig farms in the European Union was 14% (range, 0 to 46%) in breeding herds and 26.9% (range, 0 to 51%) in production herds. In addition to the baseline study, various European countries have conducted national or regional investigations to determine the prevalence of MRSA in their healthy pig herds. In Germany, investigations of the spread of MRSA in pig primary production revealed a herd prevalence of 45 to 70% (2, 42, 59). These results were higher than the 43.5% of breeding farms and 41.3% of production farms identified by the European Union. The differences might be due to the selection of farm types. German fattening farms were consistently more often MRSA positive than were breeding farms, and the number of MRSA-positive herds seemed to be correlated with pig density in the respective region. In The Netherlands, the prevalence of MRSA-positive pig herds of different production types was estimated to be 23 to 71% (15, 38, 103, 106). Particularly, farms with finishing pigs had high MRSA loads. Between 2007 and 2008, a marked increase in the percentage of Dutch MRSA-positive pig herds was noted. The upward trend was primarily attributed to the transmissibility of MRSA between distinct pig herds (15).

During further investigations on the European continent, MRSA-positive pig farms were found in Belgium (22), Croatia (49), Denmark (63), and Portugal (83) with prevalences of 16.7 to 100%. Beyond Europe, MRSA also was isolated from pigs in primary production in Canada (56, 116), the United States (70, 93), Peru (5), and several Asian countries (3, 7, 23, 55, 61, 64, 101, 111). Table 2 summarizes available publications, including respective sample sizes and detection rates.

Comparison of the molecular typing results of the MRSA isolates reveals regional differences in the dissemination of genetic variants. In Europe, Canada, the United States, and Peru, the majority of MRSA strains from pigs in primary production were assigned to CC398. Sporadically occurring non-CC398 strains were assigned to CC1, CC9, CC30, and CC97. Within the CC398 lineages, t011, t034, and t108 were the most prevalent spa types in Europe, altogether counting for 80 and 81.3% of isolated strains from European breeding and production herds, respectively (38). spa t108 was very common in The Netherlands but played a minor role in the rest of Europe. In Italy, spa t899 ST398 was the predominant clone, accounting for 27 and 24% of all isolates from pig breeding and production farms, respectively (9, 38). An exceptionally high detection rate of non-CC398 strains, particularly CC1 and CC97, was noted in the Italian pig primary production. In Canada, two human epidemic clones were identified in pig herds. CMRSA-2 (also known as USA100) accounted for 14 to 15% of the Canadian isolates. The ST5 strain was reported to be the most common cause of hospital-associated MRSA infections in humans in Canada and the most common strain found in colonized humans in the United States. CMRSA 5 (USA500) was isolated from pigs for the first time. This strain was associated with ST8, an uncommon human epidemic strain in Canada that has been regionally reported from horses (56, 116). In Asia, methicillin resistance seems to have emerged in a porcine S. aureus strain other than ST398. MRSA CC9, a minor animal MRSA sequence type in Europe and America, was predominantly isolated from swine in Thailand (5, 61), Malaysia (55, 76), China (23,
TABLE 1. Main features of the different MRSA types

<table>
<thead>
<tr>
<th>Attribute</th>
<th>LA-MRSA</th>
<th>HA-MRSA</th>
<th>CA-MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definition</td>
<td>Livestock associated: distinct strains isolated from livestock and people in close contact with livestock</td>
<td>Hospital associated: strains isolated in health care settings or from patients at least 48 h after hospital admission</td>
<td>Community associated: strains isolated in an outpatient setting or from patients within 48 h of hospital admission without risk factors for HA-MRSA</td>
</tr>
<tr>
<td>PFGE</td>
<td>Nontypeable with standard PFGE with SmaI endonuclease (11)</td>
<td>Typeable (74)</td>
<td>Typeable (74)</td>
</tr>
<tr>
<td>SCCmec</td>
<td>SCCmec types IV and V dominant (105)</td>
<td>SCCmec types I, II, and III dominant (36)</td>
<td>SCCmec types IV and V dominant (31)</td>
</tr>
<tr>
<td>MLST</td>
<td>Major clone: ST398 (38)</td>
<td>Major clones: ST8, ST250, ST239, ST247, ST5, ST228, ST22, ST36, ST45 (36)</td>
<td>Major clones: ST1, ST8, ST30, ST59, ST80, ST93 (104)</td>
</tr>
<tr>
<td>Presence of PVL genes</td>
<td>Individual isolates (118, 119)</td>
<td>Rare (31)</td>
<td>Frequent (31)</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Livestock: age, herd or farm size, holding type, animal replacement policy; use of antimicrobials is suspected</td>
<td>Prolonged antimicrobial therapy, prolonged hospitalization, care in an intensive care unit, surgical procedures, close proximity to a hospital patient who is infected or colonized with MRSA (105)</td>
<td>Gastrointestinal disease, intravenous drug use, direct contact with an individual who has a CA-MRSA skin infection, indirect contact with contaminated objects, close contact among military recruits, travel to high-prevalence areas (31)</td>
</tr>
<tr>
<td>Resistance</td>
<td>Multidrug (4)</td>
<td>Multidrug (28)</td>
<td>Often limited to β-lactam antibiotics (31)</td>
</tr>
</tbody>
</table>

*PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing.

TABLE 2. Prevalence of MRSA in the pig primary production

<table>
<thead>
<tr>
<th>Country</th>
<th>Farms</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive</th>
<th>Pigs</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive</th>
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<td></td>
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<td></td>
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<tr>
<td>Belgium</td>
<td>50</td>
<td>34</td>
<td>68.0</td>
<td>1,500</td>
<td>663</td>
<td>44.2</td>
<td>22</td>
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<td>6</td>
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<td>68</td>
<td>24</td>
<td>35.3</td>
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<td>33.3</td>
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<td>152</td>
<td>52.4</td>
<td>300</td>
<td>147</td>
<td>70.3</td>
<td>93</td>
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<td>16.7</td>
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<td>1</td>
<td>3.3</td>
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<td>7</td>
<td>22.6</td>
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<td>35</td>
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<tr>
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<td>56.0</td>
<td>209</td>
<td>147</td>
<td>70.3</td>
<td>93</td>
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<tr>
<td>The Netherlands</td>
<td>31</td>
<td>22</td>
<td>71.0</td>
<td>240</td>
<td>7</td>
<td>2.9</td>
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<tr>
<td>The Netherlands</td>
<td>171</td>
<td>115</td>
<td>67.3</td>
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<td>7</td>
<td>2.9</td>
<td>70</td>
<td>70</td>
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<td>North and South America</td>
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<td>24.9</td>
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<tr>
<td>United States</td>
<td>209</td>
<td>147</td>
<td>70.3</td>
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<tr>
<td>United States</td>
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<td>China</td>
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<td>41.9</td>
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<td>15.8</td>
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<td>0.8</td>
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<td>5</td>
<td>4.0</td>
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<td>25.0</td>
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<td>4</td>
<td>10.0</td>
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<td>70</td>
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</table>
Risk factors for the transmission of MRSA. Among the reviewed articles, 11 included examination of the effect of 26 potential risk factors on the spread of MRSA within pig primary production. Depending on the study design, risk factor analysis was performed using either univariate or multivariate statistics. Multivariate analyses revealed that pig age, herd or farm size, farm type, and animal replacement policy had a significant effect on the spread of MRSA (2, 9, 15, 39).

Risk factors for the transmission of MRSA: within-herd prevalence. The individual MRSA colonization of pigs is subject to an inner herd dynamic. Each animal can change its MRSA status within a study period, and assumptions were made about whether this change might be age related. Weaning and rehousing piglets from farrowing pens to flat decks seems to increase the frequency of MRSA detection. In a longitudinal German study, piglets on two independent farms were repeatedly tested for MRSA carriage during rearing. Grower-finisher pigs tended to have higher MRSA carriage rates than did pigs before weaning (75). Another longitudinal investigation in a Canadian antibiotic-free farrow-to-feeder pig unit confirmed the German results. During the study, the MRSA prevalence increased from 34.5% of pigs before weaning to 85% of pigs in the postweaning period (117). Possible reasons for the increase in prevalence around the time of weaning might include an age-related higher susceptibility to colonization due to specific characteristics of the commensal nasal microflora. Weaning also might facilitate MRSA colonization as a result of stress, confinement of MRSA-negative pigs with MRSA-positive pigs, or cross-contamination via the environment or human handling (117). In contrast, in an earlier Canadian study no correlation was found between MRSA status and the age of the pigs (56). Suckling piglets also seem to be at risk for MRSA colonization. In a risk factor analysis among pigs of different age groups in The Netherlands, groups of suckling piglets had MRSA detection rates almost identical to those of weanling pigs, each group with approximately 53% positive samples (15). Similar results were found in a longitudinal investigation in a U.S. pig production system. In that study, 100% of the pigs younger than 12 weeks carried MRSA. The prevalence decreased to 50% after 18 weeks, then increased to 63% until week 21, and then dropped to 36% in adult animals (93). Suckling pigs may suffer from a high burden of MRSA because of age-related underdevelopment of the commensal nasal microflora, which might enhance susceptibility to MRSA colonization (117). The frequent use of antibiotics in suckling and postweaning pigs might also contribute to the burden of MRSA in these age groups by intensifying the selective pressure (15). The prevalence of MRSA in piglets also was associated with the MRSA status of the sow before farrowing. Although MRSA is not necessarily transmitted from dam to offspring during farrowing, the probability of a piglet becoming colonized was 1.4 to 2 times higher when the sow was MRSA positive (75, 114, 117). Under experimental conditions, Moodley et al. (71) found that the transmission of MRSA ST398 from positive sows to their progeny was an efficient route for the vertical spread of MRSA.

Risk factors for the horizontal transmission of MRSA. Farm management is a decisive factor for the dissemination of MRSA, mainly influenced by herd size and production type. Intensive piggeries provide optimal conditions for the introduction and transmission of MRSA. A herd size of more than 500 pigs was identified as a risk factor for MRSA in finishing farms in Germany (2) and in breeding farms in The Netherlands (15). European breeding farms with more than 400 pigs and production farms with more than 100 animals were twice as likely to be MRSA positive than were farms with fewer than 100 pigs (39). A significant increase in prevalence was found in Bavarian pig herds with more than 1,000 animals (42) and in Italian herds of 9,000 or more pigs (9). The risk factor of herd size appears to include several underlying risk factors such as hygiene, the purchase of gilts, the number of suppliers, and antimicrobial agent use (15, 39).

Farm type was also of particular importance for the presence of MRSA. However, the investigations reviewed did not produce consistent results. The highest MRSA prevalences were identified on finishing farms (42), wean-to-finish farms (2), breeding-to-farrowing farms (17), and farrowing farms (59). MRSA was not isolated from pigs in a study of German alternative farm systems (24). In contrast to conventional fattening farms, the alternative systems did not buy animals from conventional systems, raised fewer than 600 pigs, kept them on spacious lairages with straw bedding, and did not administer antibiotics to animals larger than 25 kg. The isolated examination of production type as a risk factor for MRSA in pig herds is difficult because this variable might be correlated with other factors. For example, farm types differ in the purchasing frequency of pigs and in the number of suppliers, both potential risk factors for the introduction of MRSA into farms (2). Although a correlation between routine use of antimicrobials and prevalence of MRSA in livestock animals has been assumed repeatedly (30, 93, 106), the association has not been confirmed for pig husbandry based on multivariate analyses (2, 15, 17). The only significant association has been found in a population subgroup of 16 Bavarian closed production systems. Pigs treated with up to four different
antimicrobials were less often colonized by MRSA than were pigs that received more than four different antimicrobials during rearing (42).

In an in vivo experiment, a short treatment with tetracycline significantly increased the MRSA counts in nasal samples of piglets compared with nontreated controls. However, the extent to which the amount of MRSA in pig nasal passages is related to the spread of MRSA is not clear (72).

**Risk factors for the transmission of MRSA: between-herd transmission.** Animal trading and transportation is an important factor in the spread of MRSA. Once introduced into the pig population, intensive trade relations accelerate the dissemination of MRSA among herds. The number of MRSA-positive farms with breeding pigs is significantly associated with the number of pigs imported into the country. Importation of pigs from countries with a high MRSA prevalence rate increases the risk of MRSA introduction (39). Farms with open production system are at risk of introducing MRSA into the stock by purchasing MRSA-colonized animals. Farms using MRSA-positive suppliers are 11 times more likely to become MRSA positive than are farms that buy pigs from MRSA-free suppliers (17).

During transportation to the abattoir and during the waiting period in lairages before slaughter, transmission of MRSA between pigs from different farms can occur. By combining prevalence rates of 10 pig herds at the farm and at the slaughterhouse before stunning, a longitudinal study in the United States revealed that animal transportation leads to an increase in MRSA colonization from 2.9% at the farm level to 11.3% at the slaughterhouse (70). In a Dutch experimental study (16), four MRSA-negative pig herds were transported to the slaughterhouse. Based on the results of nasal swabs taken on arrival at the abattoir, 10.3% of the pigs from two of the batches became MRSA positive during transport. Repeated sampling after stunning revealed an increase in MRSA prevalence up to 59.8% after the pigs had spent their resting time in the slaughterhouse lairages. MRSA-positive animals were found in all batches at prevalences of 6.7 to 100% in each batch. Only one truck picked up other pigs on the way to the slaughterhouse, and the animals were housed in separate sections of the truck and lairage; therefore, the MRSA must have been transmitted directly by animal contact and indirectly through cross-contamination via environmental factors. Truck drivers and abattoir personnel might also serve as vectors.

### MRSA PREVALENCE AT SLAUGHTER AND MEAT PROCESSING

When MRSA is present in the pig population, the delivery of colonized animals to the slaughterhouses is inevitable. As a commensal, MRSA can colonize pigs without any clinical symptoms. Without microbiological screening, it is not possible to distinguish between MRSA-positive and -negative herds to allow logistical slaughter.

Various investigations have been conducted to evaluate to what extent MRSA enters the slaughterhouses via colonized pigs. Samples have been taken from pigs at slaughterhouse lairages after stunning. However, data collected at the beginning of the slaughter process cannot be used directly to infer MRSA prevalence in the primary production sector because cross-contamination during transport or in slaughterhouse lairages can raise the MRSA detection frequency in the herds.

Denmark (11), Germany (10, 54, 99), Italy (9), Switzerland (51, 80), Spain (48, 84), and The Netherlands (30) have reported prevalences of MRSA-positive pigs of 1.3 to 64.7%. Other investigations have been conducted on Tenerife (73) and in Asia (7, 23, 64). The results of the available publications are summarized in Table 3.

The regional distribution of spa types identified at stunning was similar to those observed in the primary production. Most of the porcine MRSA strains at European

<table>
<thead>
<tr>
<th>Country</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive</th>
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**TABLE 3. Prevalence of MRSA among pigs at the beginning of the slaughter process**
Abattoirs belonged to *spa* types t011, t034 and t108. The latter strain was primarily identified by Dutch and Spanish investigations, accounting for 37.5% (30) and 11% (48, 84) of all isolates, respectively. *spa* type t899 was predominating in Italian abattoirs and could be found in 49% of the MRSA-positive slaughter pig herds. In addition, 47% of the positive herds carried non-CC398 strains (CC1, CC9 and CC97) (9). Spain also investigated the MRSA prevalence among slaughter pigs on the island of Tenerife and identified 85% positive animals (73). ST398 was exclusively isolated. Increased MRSA transmission rates in consequence of geographical characteristics of the Island were made responsible for the high MRSA prevalence. Due to the narrowness of the territory pigs have to be raised under intensive housing conditions, which might facilitate the within-herd spread of MRSA. Cross-contamination during transportation and in lairages at the sole slaughterhouse of the island might have also contributed to an interherd transmission.

In Asia, MRSA was reported among slaughter pigs in Korea (64), China (23), and Japan (7) with low prevalences of 0.9 to 7%. Korea was the only Asian country that reported CC398 as the dominant strain in the regional pig population; 81% of the examined slaughter pigs carried CC398 strains. The rest of the isolates were assigned to ST72, a human-associated strain. The Chinese slaughterhouse study confirmed *spa* t899, multilocus ST9 as the dominant sequence type in the Chinese pig population (23). One pig sampled in a Japanese abattoir was positive for MRSA t002, which was assigned to ST221 and CC5 (7).

**Transmission of MRSA along the slaughter line.** Slaughter and meat processing play important roles in the transmission of MRSA from pig to pork. Figure 1 shows a flow diagram of the essential steps of the pork production chain. The high level of entry of MRSA into the slaughterhouses via colonized pigs raises questions of how and to what extent MRSA spreads along the process chain. Three studies have included an analysis of the prevalence of MRSA at different stages of the pork production chain (10, 54, 70). Although differences in the study designs reduce the validity of result comparisons, all investigations revealed notable reduction of MRSA along the meat processing chain. Although 11.3 to 64.7% of the pigs carried MRSA at stunning, the prevalence decreased to 2.8 to 3.8% in the final products. Figure 2 compiles evaluated prevalences separately for each process step. Beneke et al. (10) took samples at several stages of the fresh pork production process in a German abattoir and found a successive reduction in MRSA prevalence along the chain. Kastrup (54) examined the spread of MRSA in five German abattoirs. She found higher carrier rates based on samples at the slaughter line than when samples collected at stunning were analyzed. Molla et al. (70) reported a decreasing prevalence along the slaughter line with a subsequent increase in the final meat products. The observed differences in the course of each process along the chain indicate that the individual hygiene performance of the corresponding abattoir may have a greater influence on the MRSA status of the final product than the carriage rate in the delivered animals. The abattoir with the highest MRSA burden at stunning had the lowest burden in their processed meat.

The numbers of studies tracing MRSA along the slaughter line is limited. Findings about the influence of single slaughter steps on microbiological carcass contamination and decontamination in general and the burden of *S. aureus* in particular could be applied to analyses of MRSA and used to draw conclusions about MRSA behavior during pig slaughter.

Several investigations that included tracing of total bacterial counts, *Enterobacteriaceae*, or other indicator organisms along the slaughter line revealed that scalding is the first slaughter process with the potential to reduce the amount of bacteria on pig carcasses. Remaining microorganism on carcasses predominantly are thermoduric and gram positive (13, 43, 44, 77, 81, 86, 94, 100). The extent of

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**FIGURE 1.** Process flow diagram of pork production chain.
microbial reduction depends on both the time and temperature conditions and the heat resistance of each microorganism. Scalding treatments usually are carried out at 60 to 62 °C for 6 to 8 min (14, 95). Because S. aureus has a D55-value of approximate 66 s, a significant reduction of MRSA during scalding is expected (12). Gill and Bryant (44) found that staphylococci can be consistently detected in minor amounts during successive processing steps. An analysis of the effect of certain production steps on the quantity of coagulase-positive S. aureus (CPS) on pig carcasses was conducted recently in two Swiss abattoirs (97). After bleeding, CPS was isolated from 96 to 100% of all carcasses, with counts of 2.5 to 3.5 log CFU cm⁻². Scalding for 5 and 8.5 min at 59 to 62°C reduced the number of CPS-positive carcasses to 18 and 20%, respectively, with counts around 1.0 log CFU cm⁻². Although one abattoir was able to maintain a low level of CPS contamination along the slaughter line, the proportion of CPS-positive carcasses at the second abattoir increased, with a proportion of 99% at the end of the line. Recontamination could mainly be attributed to the combined dehairing and singeing operations. The considerably different recontamination levels noted at these two abattoirs emphasizes the importance of effective hygiene strategies (97).

In general, dehairing is a critical processing step for cross-contamination during pig slaughter (44, 45, 77, 81). The mechanical treatment of the carcass with rotating scrapers and rubber flails leads to increased dissemination of porcine bacteria from mouth, nose, skin, and intestinal tract. While progressing through the dehairer, the scalded carcasses can get contaminated by the detritus that accumulates in the machine. Conventional dehairing equipment is difficult to clean, and when sanitization is insufficient a persistent microbiological flora can become established (86, 97). Hot water of 60 to 62 °C that was sprayed on the carcass as it moved through the dehairer reduced the surface contamination (94, 95). Low concentrations of MRSA or other S. aureus strains in scalding water and the infrequent detection of MRSA-positive samples indicate a low impact of scalding water on cross-contamination of MRSA during the slaughter process (54, 96).

Singeing has been reported to decontaminate the surface of pig carcasses significantly, but published quantitative data differ widely. Using conventional automatic singeing systems with a passage of 10 to 15 s at 900 °C, a 2.5- to 3-log reduction in total bacterial counts is achievable (13, 14, 77, 81). In contrast, some investigators reported that singeing had no effect on the microflora or even increased surface contamination; however, these results might be due to antiquated facilities or hygienic deficiencies (33, 97).

Various researchers indicated that the reduction in microorganisms achieved by singeing is frequently reversed by polishing. Polishing systems work with scrapers and nylon brushes, which are difficult to clean and therefore accumulate porcine microorganisms (77, 81, 94, 120). Depending on the singeing system used, certain sectors of the carcass might be insufficiently exposed to flaming, and surviving bacteria could be redistributed over the carcass during polishing (14, 44). The extent of recontamination with MRSA during polishing seems to depend on the cleanliness of the polisher and the effectiveness of the singeing process.

In various investigations, an increased amount of fecal bacteria was detected on the surface of carcasses after evisceration, and the intestinal tract was identified as the main source of contamination at this processing stage (81, 86, 97, 120). Because staphylococci and their resistant variants can regularly be isolated from porcine intestines (98), transmission from the intestines to muscle tissue can
also be expected. Postevisceration spraying with water is used to remove visible contaminants from carcasses before they enter the chiller. Investigations revealed that using water of 85°C for 20 s can decrease existing carcass contamination, whereas cold water merely distributes existing bacteria over the carcass surface (13, 46, 66).

Contrasting results regarding the effect of slaughter processes on superficial Staphylococcus aureus contamination of swine carcasses were obtained in hog slaughter plants in Iowa. The percentage of S. aureus–positive swabs increased linearly from 4.4% after singeing and polishing to 12.6% after 24 h of carcass chilling, and this increase was attributed to the increase in human handling at advanced slaughter stages (89). Investigations in a Brazilian abattoir did not reveal any significant influence of slaughter processes on the level of S. aureus isolated. Surface swabs were taken from carcasses after dehairing, before and after evisceration and splitting, and after 24 h of refrigeration. Bacterial counts of 1.2 to 1.5 log CFU cm$^{-2}$ were obtained at each sampling point. Although the investigated slaughter line involved decontamination steps at which the carcasses were sprayed with 1.5 to 2.0 ppm of chlorine water and 0.85 to 3% lactic acid, no significant effect on the burden of S. aureus was noted (29).

To improve the microbiological status of pig carcasses at the end of the slaughter line, additional antimicrobial intervention technologies have been proposed. The application of hot water, steam, organic acids, chlorine, or various salts to porcine carcasses can reduce bacterial counts by as much as 2 log units (66). In accordance with article 3(2) of Regulation 853/2004 of the European Parliament laying down specific rules on the hygiene of foodstuffs (37), the decontamination of meat in abattoirs of the European Union is limited to the use of drinking water.

To inhibit proliferation of residual MRSA on carcasses, the surface temperature must be reduced as rapidly as possible. Under the terms of Regulation 853/2004, the temperature of the complete carcass must be reduced below 7°C before further processing unless the slaughter premises include a separate cutting area (37). In the pork industry, pig carcasses are usually chilled overnight using either conventional single stage chilling regimes or alternative cooling technologies such as spray chilling, ice bank chilling in humid air at 2°C, and rapid or ultrarapid chilling, where carcasses are initially exposed to a prechilling period with air at −10 to −30°C (18, 52). S. aureus strains that originate from pigs or the environment do not grow at temperatures below 6°C (12). Spescha et al. (97) found that a 77% decrease in the proportion of S. aureus–positive carcasses and a quantitative reduction of S. aureus on the surface of carcasses were achieved after chilling. Freeze chilling (at temperatures of −10 to −25°C for 45 to 60 min, followed by chilling at 2°C for 23 h) reduced S. aureus by 1 log CFU cm$^{-2}$ on untrimmed carcasses (20).

Meat processing. After carcasses leave the slaughterhouse chillers, residual MRSA on carcass surfaces can be transmitted during further processing via human hands, cutting tools, and any surfaces with direct meat contact. The increase in manual handling during processing also can facilitate the entry of human MRSA strains into the production units. A Swiss meat processing plant reported the presence of S. aureus on 22.7% of the received chilled pork hindquarters from 18 European suppliers, with bacterial counts of 0.1 to 2 log CFU cm$^{-2}$ (92). The contaminated pork was traced back to few specific abattoirs, confirming that the burden of staphylococci on pork is influenced by the slaughter process.

A significant reduction of the initial MRSA counts on meat loins can result from the removal of surface tissue during the trimming procedure (89). While investigating German pork processing units, Kastrup (54) determined a MRSA detection frequency of 6% on meat trimmings, 2% on processing equipment, and 5% on employees. Because the detection of MRSA-positive meat trimmings was always connected with MRSA-positive environmental or hand swabs, transmission of MRSA along the line was expected. Molecular typing of the isolates confirmed this suspicion; all MRSA from meat, plant environment, and hands were identified as spa t011. With 4.2% MRSA-positive meat samples, Beneke et al. (10) obtained a similar detection rate in the processing area of a German abattoir. MRSA-positive environmental swabs were obtained only in the slaughter area, whereas swabbed processing utensils did not contain MRSA. In general, S. aureus remains viable on stainless steel surfaces and hence might present a recontamination hazard for considerable periods of time. In an experimental setting, S. aureus at a contamination level of 5 to 7 log CFU/100 cm$^2$ was detectable on dry stainless steel for at least 96 h. Even at lower initial levels of 3 log CFU/100 cm$^2$, S. aureus could be isolated within 48 h (60). In The Netherlands, de Jonge et al. (27) assessed the presence of MRSA in three meat processing facilities and two institutional kitchens. MRSA was not isolated from any human nose or hand swabs, but 31 participants (33%) carried methicillin-susceptible S. aureus. Five meat samples (14.3%) were contaminated with MRSA. The selection of analyzed meat contained 10 pork samples, 2 (20%) of which were positive for MRSA spa t011.

Final product. When MRSA is present in the pig population of a country, it can also be recovered from corresponding final products at retail. Table 4 gives an overview of published investigations of the MRSA prevalence among pork and pig meat products.

In Europe, MRSA-positive pork and pig meat products were reported from Denmark (1), Germany (10, 19, 54, 91), Spain (68), and The Netherlands (26, 107), with prevalences of 1.8 to 15.8%. Combining the MRSA detection rates of different final product types, minced pig meat portions were twice as likely to be MRSA positive than were fresh pork samples; this difference could be due to the processing method (19, 91). Because minced meat is usually made of meat from several animals, the probability of any MRSA entry increases with the number of carcasses used when carcass contamination rates are assumed to be constant. Mincing of meat is associated with an increase in surface area, which might improve growth conditions for S. aureus.
TABLE 4. Prevalence of MRSA among pork and pig meat products

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In accordance with the regional spa type distribution in primary production and at slaughterhouses, most of the MRSA isolates could be assigned to spa t011 and t034. spa t108 was found only among Dutch food samples. MRSA-positive samples from pork imported into Denmark indicated the presence of MRSA in other European countries, such as Poland and France (1). In the United States (50, 78, 85, 112, 113, 115) and Canada (113, 115), MRSA was isolated from 3.6 to 9.6% of pig meat products. The majority of MRSA strains in U.S. pork products belonged either to the widespread hospital-acquired sequence type USA100 (ST5) or to USA300 (ST8), which is the most common CA-MRSA strain in the United States. ST398 was not widespread in U.S. meat. The reviewed investigations indicate that MRSA in the U.S. pork chain is probably the result of human contamination (50, 85, 112). No significant difference in the prevalence of MRSA was observed when comparing pork samples from animals raised on conventional farms and those from animals raised on alternative farms without antibiotics (78). Three main MRSA clones were identified in Canada after molecular typing. Most of the MRSA were identified as Canadian epidemic MRSA-5 and MRSA-2, which are human-associated strains corresponding to ST8/USA300 and ST5/USA100, respectively. Only a minor proportion of isolates were identified as spa t034, assigned to CC398 (113, 115). Results from a quantitative MRSA analysis revealed that 60% of the MRSA-positive samples had levels of 1.3 log CFU/g. The remaining contamination was 1.5 to 3.6 log CFU/g. In contrast to the European findings, the Canadian pork chops were twice as likely to be MRSA positive than the sampled minced meat portions (113). In Asia, MRSA was reported on retail pork from Korea and Hong Kong with prevalences of 7.1 and 21.5%, respectively (65, 79). According to results at Chinese abattoirs, Chinese pork products sampled at Hong Kong markets predominantly carried MRSA of spa t899, assigned to ST9 (79). In Korea, MRSA ST72 was exclusively recovered from pork at retail (65). Because ST398 is the dominant type in the Korean pig population (64) and ST72 is the most prevalent type of CA-MRSA among humans in Korea (8), contamination during processing via staff members is the most probable explanation for the ST72 recovered from the retail pork samples.

PUBLIC HEALTH RELEVANCE

Results of several investigations have verified that the presence of LA-MRSA on pig farms constitutes a substantial health risk for farmers and veterinarians who come into contact with colonized animals, their excretions, and contaminated dust (25, 102). Several researchers have found that MRSA CC398 can cause serious diseases such as endocarditis, pneumonia, and urinary tract, wound, and soft tissue infections (6, 34, 67, 87). The incidence of CC398 detection in hospitals and the proportion of MRSA infections caused by LA genetic types seems to be correlated with the regional density of livestock farming (107, 109). In Germany, the proportion of LA-MRSA among MRSA strains isolated from humans is increasing (90).

The wide dissemination of LA-MRSA in pig meat products has been demonstrated, but the public health relevance of contaminated meat remains unclear. MRSA colonisation via handling or consumption of contaminated food seems to be very rare but not impossible. Only two clinical MRSA outbreaks have been traced to the consumption of contaminated meat, but both incidences were associated with non-CC398 strains. In the first case, a severely immunocompromised patient suffered from septicaemia after ingestion of MRSA-contaminated food. The causative MRSA strain was subsequently transmitted to several other patients via a colonized nurse (57). The second incidence was a typical food intoxication caused by cole-slaw, which was contaminated with toxin-producing MRSA strains (53). Investigations among professional meat handlers in The Netherlands revealed that even high-frequency exposure resulted in a low colonization rate of MRSA-contaminated dust (37). The substantial health risk for farmers and veterinarians who come into contact with LA-MRSA on pig farms constitutes a considerable risk factor for human infection. Key points for the control of LA-MRSA transmission are proper hygiene and standard recommendations for hygienic handling and sufficient heating of raw meat are followed, the risk should be greatly reduced if not eliminated.

The number of LA-MRSA strains in meat might be another reason for the restricted transmission rate. Reliable quantitative data concerning LA-MRSA in pork and pig meat products are not available, although some evidence suggests that the number of MRSA strains in meat is low. In a Canadian quantitative study of different types of retail meat, researchers identified low levels of Canadian epidemic MRSA-2, with 37% of samples below the detection threshold (113); most quantifiable samples contained <2 log CFU/g. During quantitative investigations...
in The Netherlands, the isolation of MRSA from meat products was possible only when a sensitive preenrichment protocol was used (108). However, permanent MRSA colonization or infectious disease from consumption or handling of MRSA-contaminated meat may be possible; the required infection dose has not yet been determined. Another reason for the discrepancy between the high detection frequency of MRSA CC398 and the low number of infectious disease cases caused by this type of strain might be the lack of clinically important virulence factors (4, 59). Although the burden of infectious diseases associated with LA-MRSA has been low, continual surveillance is important because the pathogenicity potential of CC398 can evolve by insertion of additional genes. In China, five PVL-positive MRSA ST398 isolates were associated with lung and wound infections in hospitalized patients (119). The Robert Koch Institute (88) recently reported two PVL-positive methicillin-susceptible ST398 isolates from cases of recurrent furunculosis in Germany. In Italy, a worker at a dairy farm suffered from severe sepsis due to infection with MRSA ST398, and although the isolated strain did not harbor PVL-encoding genes, its virulence resembled that of PVL-positive strains.

CONCLUSIONS

MRSA has been isolated at consecutive steps in the pork production chain. Because longitudinal interventions are rare, results of various prevalence studies conducted with the same regional and temporal parameters were used to draw conclusions about the dynamics of MRSA spread along the pig processing line. However, differences in study design limit the comparability of the results. To classify pig herds as MRSA positive, a variable number of environmental dust samples, nasal swabs, perianal swabs, or a combination of these samples was used. Investigations at the retail level included samples of different numbers of pork and pig meat products of variable weight, which were analyzed either directly after one or two enrichment steps or indirectly using swab or rinse samples. The use of different laboratory protocols for MRSA isolation and identification, antimicrobial susceptibility testing, and molecular characterization of the strains additionally hamper result comparisons across studies. Despite all these differences, the results of the reviewed investigations were similar in the considerable decrease in the detection frequency of MRSA along the chain from pigs at stunning to the final retail product.

Pig herds are an important reservoir for MRSA. Animal age, herd or farm size, farm type, and animal replacement policy have a significant influence on the transmission of MRSA within and between the herds. Farm level sampling in general can provide precise information about the epidemiology of MRSA in pig primary production. However, because of small sample sizes, most of the reviewed investigations only provided evidence of a porcine MRSA reservoir and the presence of different genetic variants in the different countries. The national prevalence and diversity of MRSA in swine herds cannot be assessed on the basis of most available data sets. Sampling of pigs at the abattoir before or shortly after stunning is an appropriate way to evaluate the full extent of MRSA entry into the slaughter process. However, conclusions cannot be drawn directly about the prevalence in the pig population as whole because MRSA transmission during transport and in lairages may occur.

With the delivery of MRSA-positive pigs to the abattoirs, the pathogen is able to enter the food chain. Because of the absence of specific clinical symptoms, MRSA-positive animals cannot be identified and separated from other slaughter animals to reduce cross-contamination. Nevertheless, the standard pig slaughter process seems to reduce MRSA contamination along the processing line. Steps such as superficial heat treatments by scalding and flaming can significantly diminish the amount of MRSA on carcasses. However, residual MRSA can become redistributed over the carcass during dehairing and polishing via surface treatment machinery. Recontamination might also occur via fecal contamination at evisceration. The increase in manual handling during processing facilitates the entry of human MRSA strains into the production units. Molecular characterization of MRSA strains isolated along the production chain revealed regional differences in the distribution of genetic clones. Because identical MRSA clones are predominant both in pigs at farm or at slaughter and in retail pork, MRSA on final pig products seems to originate from animal sources and is transmitted along the pork production chain.

The slaughter process must be evaluated to identify critical steps for MRSA transmission. By optimizing processes for carcass decontamination and avoiding recontamination by implementation of effective cleaning and personal hygiene management, MRSA transmission from animal to meat products can be minimized.

ACKNOWLEDGMENT

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