Low prevalence of non-typable Methicillin-resistant *Staphylococcus aureus* in meat products in The Netherlands


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**Abstract**

Recently, a new clone of methicillin resistant *Staphylococcus* (*S.*) *aureus* (MRSA) emerged in the Netherlands that was related to pig farming. A survey in pigs showed that nearly 40% carried this new clone. This new type is characterised by being untypable with pulsed field gel electrophoresis (PFGE). This study was undertaken to determine the prevalence and genetic relationship of *S. aureus* and MRSA in meat products. Samples were collected between February and May 2006. A total of 79 raw pork and cow meat products were randomly collected from 31 different shops (butchers n=5, supermarkets n=26) in the South of The Netherlands. The samples were cultured using three procedures. Identification of the strains as *S. aureus* and methicillin resistance were determined by Martineau PCR assay for species identification and PCR for the presence of the *mecA* gene. Susceptibility to cefoxitin and doxycyclin was determined using disk diffusion according to CLSI standards. All isolated *S. aureus* strains were genotyped by amplified fragment gel electrophoresis (AFLP). Direct inoculation of plates yielded no MRSA positive isolates. The first enrichment broth yielded 30 *S. aureus* isolates. One *S. aureus* isolate in pork meat was identified as MRSA. With the addition of the double-enrichment broth culture system another 6 *S. aureus* were detected, one of which was methicillin resistant. Combining the results of both enrichment broth culture procedures, in total 36 *S. aureus* isolates were obtained from 34 samples. Two isolates from pork meat (2.5% of total samples) were found to be methicillin resistant. A total of 19 shops (61%) were found to be positive for *S. aureus* in at least one meat sample. AFLP typing showed 8 genetic lineages, covering 81% (29/36) of the isolated strains, and a smaller number of unique sporadic isolates (20% (7/36) of isolated strains (figure 2). In 5 out of 6 shops (83%), in which more than one *S. aureus* isolate was found, there was evidence of clonal relationship between the strains of particular shops. PFGE typing of the MRSA isolates showed that 1 MRSA isolate was nontypable using *smal* digestion and identical to isolates found in pigs. The other MRSA isolate was identical to the USA 300 clone.

In conclusion, this is the first report of MRSA prevalence that is available for meat products in the Netherlands. 2.5% of the meat contained MRSA. Furthermore, *S. aureus* is found regularly in low amounts in meat. Considering the low amounts of contamination these findings suggest that under normal conditions meat consumption is very unlikely to be a hazard to consumers for the acquisition of MRSA.

**Introduction**

In 2003 a new clone of methicillin resistant *Staphylococcus aureus* (MRSA) emerged in the Netherlands that was related to pig and cattle farming [1,2]. A survey in pigs showed that nearly 40% carried this clone [2]. The detection of this strain was relatively easy since it is non-typable (NT) with Pulsed Field Gel Electrophoresis (PFGE), the method that is used for surveillance of MRSA at the National Reference Centre for MRSA (National Institute of Public Health and the Environmental, Bilthoven, The Netherlands). Further typing of NT-MRSA showed that almost all strains belonged to one MLST cluster, ST 398. This study was undertaken to determine to what extend *S. aureus* and more specific MRSA was present in Dutch meat products.
Materials and methods

Samples of various meat products from pigs and cattle, obtained from local supermarkets and butcheries, were examined for contamination with methicillin susceptible S. aureus (MSSA) and MRSA. A total of 79 raw meat products (pork n=64 and beef n=15) were collected from 31 different shops (butcheries n=5, supermarkets n=26) in the period February - May 2006. From 14 shops 1 sample was investigated, from 6 shops 2 samples, from 4 shops 3 samples, from 3 shops 4 samples, from 2 shops 5 samples, from 1 shop 9 samples and from 1 shop 10 samples. A small portion of the meat products (mean 7.9 g +/- sd 3.97) was plated directly on a chromogenic screening medium for the detection of MRSA (MRSA ID; bioMérieux, La Balme Les Grottes, France) and put into 5 ml enrichment broth, containing Mueller Hinton broth with 6.5% NaCl. After 24 h incubation at 35°C the enrichment broth was subcultured on Columbia agar plates with 5% sheep blood (CA), an MRSA-ID plate and 1 ml of the enrichment broth was put into a second enrichment broth containing phenolred mannitol broth with cefitoxin (5 μg/ml) and aztreonam (7.5 μg/ml) (Regional Public Health laboratory, Groningen, The Netherlands). The second enrichment broth was subcultured on CA and MRSA-ID. All plates were incubated 48 h at 35°C. Presumptive S. aureus colonies were confirmed with a latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd., Dartford, England), a tube coagulase test with rabbit plasma and DNase (DNase S. aureus) (Oxoid Ltd., Basingstoke, England). Species identification was confirmed by Martineau PCR. Confirmation of methicillin resistance was performed with mecA gene PCR. Susceptibility to cefoxitin and doxycyclin was determined using disk diffusion according to CLSI (formerly NCCLS) standards [3]. All isolated S. aureus strains (MSSA and MRSA) were genotyped by Amplified Fragment Gel Electrophoresis (AFLP).

Results

Direct inoculation of plates yielded no MRSA positive isolates (table 1). The first enrichment broth yielded 30 S. aureus isolates, 25 of which were detected in pork meat and 5 in beef meat. In one pork sample 2 phenotypically different S. aureus isolates were found. One S. aureus isolate in pork meat was identified as MRSA. With the addition of the double-enrichment broth culture system another 6 S. aureus were detected, one of which was methicillin resistant. Combining the results of both enrichment broth culture procedures, in total 36 S. aureus isolates were obtained from 34 samples (table 1). Twenty-seven (42.2%) pork samples and 5 (33.3%) beef samples harboured S. aureus. Two pork samples yielded 2 phenotypically different S. aureus isolates. Two isolates from pork meat (2.5% of total samples) were found to be methicillin resistant. A total of 19 shops (61%) were found to be positive for S. aureus in at least one meat sample. The range of S. aureus positive samples for each store is shown in figure 1.

AFLP typing showed 8 genetic lineages, covering 81% (29/36) of the isolated strains, and a smaller number of unique sporadic isolates (20% (7/36) of isolated strains. From the 2 samples that contained 2 phenotypically different strains, the 2 strains from 1 sample (number 31-1 en 31-2) belonged to the same lineage and the other sample contained 2 strains (number 17-1 en 17-2) belonging to 2 different genetic lineages. In 5 out of 6 shops (83%), in which more than one S. aureus isolate was found, there was evidence of clonal relationship between the strains of particular shops. PFGE typing of the MRSA isolates showed that 1 MRSA isolate was nontypable using smal digestion and identical to isolates found in pigs. The other MRSA isolate was identical to the USA 300 clone.

Discussion

This is the first survey investigating the presence of MSSA and MRSA in meat products in the Netherlands. Two meat samples (2.5%) contained MRSA. Furthermore, S. aureus is found regularly in low amounts in meat as it is sold to consumers. The prevalence of S. aureus in meat products was found to be 4%, 22.7% and 65% in 3 other studies performed in Egypt, Switzerland and Japan, respectively [4-6]. Contamination of the meat products could be traced back to certain abattoirs in Switzerland and poor hygienic and sanitary conditions in Egypt. The high rate of clonal relatedness of different strains within particular shops indicates cross-contamination of the meat at some point during processing. Therefore, the strain in the sample is not necessarily indicative of the strain that was carried by the animal at the source.
This study demonstrates that MRSA has entered the food-chain. As the amounts were very low it is not likely to cause disease, especially if meat is properly prepared before consumption. However, contamination of food products may be a potential threat for the acquisition of MRSA by the person who handles the meat and, even worse, foodborne illness by MRSA. Both events have been previously described. Foodborne disease caused by contamination of pork meat with MRSA was caused by a food handler who was carrier of MRSA with the same PFGE pattern as several cases [7]. Contamination of food products was the transmission route for an MRSA outbreak on a hospital ward in Erasmus MC in Rotterdam, The Netherlands [8]. A dietary worker carried MRSA in his throat and transmitted MRSA via food to patients. One immunocompromised patient was likely infected this way, developed a severe sepsis and died.

All reports of MRSA in meat-products described before dealt with MRSA from human origin that was contaminating the meat. In this report the NT-MRSA in the meat is from animal origin.

Although in this study the pig-related MRSA strain was found in only one product and in very low amounts, it does show that MRSA has made its way into the food chain.

References

Table 1. Number of MSSA and MRSA in pork and beef meat, separated for the different culture systems.

<table>
<thead>
<tr>
<th></th>
<th>Direct culture</th>
<th>Single enrichment broth</th>
<th>Single and double enrichment broth</th>
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<tbody>
<tr>
<td></td>
<td>Total no. of samples</td>
<td>MSSA no. of strains</td>
<td>MRSA no. of strains</td>
</tr>
<tr>
<td>Pork</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Beef</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>0</td>
<td>0</td>
</tr>
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Figure 1. Number of positive samples per shop.