Short communication

High prevalence of methicillin resistant *Staphylococcus aureus* in pigs

A.J. de Neeling\textsuperscript{a,*}, M.J.M. van den Broek\textsuperscript{b}, E.C. Spalburg\textsuperscript{a}, M.G. van Santen-Verheuvel\textsuperscript{a}, W.D.C. Dam-Deisz\textsuperscript{a}, H.C. Boshuizen\textsuperscript{a}, A.W. van de Giessen\textsuperscript{a}, E. van Duijkeren\textsuperscript{c}, X.W. Huijsdens\textsuperscript{a}

\textsuperscript{a}National Institute for Public Health and the Environment, Bilthoven, The Netherlands
\textsuperscript{b}Food and Consumer Product Safety Authority, Zutphen, The Netherlands
\textsuperscript{c}Veterinary Faculty, Utrecht University, Utrecht, The Netherlands

Received 17 November 2006; received in revised form 25 January 2007; accepted 26 January 2007

Abstract

Recently methicillin resistant *Staphylococcus aureus* (MRSA) was isolated from pigs and pig farmers in The Netherlands. In order to assess the dissemination of MRSA in the Dutch pig population, we screened 540 pigs in 9 slaughterhouses, where a representative portion of Dutch pigs (63\%) was slaughtered in 2005. We found 209 (39\%) of the pigs to carry MRSA in their nares. Forty-four of 54 groups of 10 consecutive pigs (81\%), each group from a different farm, and all slaughterhouses were affected.

All MRSA isolates belonged to 1 clonal group, showing Multi-Locus Sequence Type 398 and closely related \textit{spa} types (mainly t011, t108 and t1254). Three types of the Staphylococcal Chromosome Cassette (SCC\textit{mec}) were found: III (3\%), IVa (39\%) and V (57\%). All 44 tested isolates (1 isolate per group) were resistant to tetracycline, reflecting the high and predominant use of tetracyclines in pig husbandry. Twenty-three percent of the isolates were resistant to both erythromycin and clindamycin and 36\% to kanamycin, gentamicin and tobramycin but only a single isolate was resistant to co-trimoxazole and none to ciprofloxacin and several other antibiotics.

The percentage of MRSA positive pigs was significantly different among slaughterhouses and among groups within slaughterhouses, indicating a high prevalence of MRSA in pigs delivered from the farms as well as cross contamination in the slaughterhouses.

\textsuperscript{*}Corresponding author at: National Institute for Public Health and the Environment (RIVM), Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, pb 22, Antonie van Leeuwenhoecklaan 9, 3721 MA Bilthoven, The Netherlands.
Tel.: +31 30 2742729; fax: +31 30 2744418.
E-mail address: Han.de.Neeling@rivm.nl (A.J. de Neeling).

0378-1135/$ – see front matter \textcopyright 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2007.01.027

1. Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) in humans is still rare in The Netherlands. Last year 2\% of the *S. aureus* isolates from hospitals were resistant to
oxacillin (SWAB, 2006) and only a small proportion (0.03%) of patients admitted to hospitals carried MRSA (Wertheim et al., 2004). Occasionally MRSA has been cultured from dogs, cats and diseased horses, but no MRSA was found in a survey of 200 healthy horses in The Netherlands (Busscher et al., 2006).

Recently Voss et al. (2005) isolated MRSA from three patients who had contact with pigs. These authors also tested 26 pig farmers. Six of them (23%) carried MRSA. Subsequently MRSA was isolated from several members of a family living on a pig farm and 8 out of 10 pigs at the same farm carried MRSA (Huijsdens et al., 2006). All MRSA-isolates from human and porcine origin in these investigations were non-typeable by standard Pulsed-Field Gel Electrophoresis (PFGE) using the SmaI restriction enzyme (NT). The NT MRSA contain a restriction modification enzyme, which methylates the SmaI-recognition sequence (Bens et al., 2006).

These observations prompted us to determine the prevalence of MRSA in healthy pigs in nine Dutch slaughterhouses. We further analyzed the porcine MRSA by molecular typing and susceptibility testing.

2. Materials and methods

2.1. Survey in nine slaughterhouses

From November 2005 to January 2006 in each of nine slaughterhouses all over The Netherlands 6 groups, 10 pigs per group, 540 pigs in total, were screened. In 2005, 63% of the pigs raised in The Netherlands were slaughtered in the nine investigated slaughterhouses. In The Netherlands each slaughterhouse buys pigs from a broad range of farms, with few exclusive contracts between a slaughterhouse and the farms supplying pigs. So we are confident that we have screened a representative sample of the pigs in the Dutch slaughterlines. A group consisted of 10 consecutive pigs in the slaughterline, each group from a different farm, except 1 group, which was composed of pigs from 3 farms and 2 groups, which were both from 1 farm.

A swab (Medical Wire & Equipment Co. (Bath) Ltd. Corsham, Wiltshire, no. MW102) was taken from the nares of the pigs just after stunning, by officials of the Dutch Food and Consumer Product Safety Authority (VWA). Within 5 h after sampling, swabs were transferred into tubes containing 5 mL Phenol Red Mannitol Broth (Brunsorschwig Chemie, Amsterdam) with 4 mg/L oxacillin (Sigma) and 75 mg/L aztreonam (ICN). After 18 h incubation at 35 °C, the bacteria from each tube were plated onto sheep blood agar and three selective agar media: MRSA Select Agar (BioRad, Veenendaal), Oxacillin Resistance Screening Agar and Chromogenic MRSA Agar (Oxoid, Haarlem).

After 18 h incubation at 35 °C, suspected colonies were plated onto sheep blood agar and incubated for 18 h. Colonies suspect of being MRSA were tested by PCR for the S. aureus specific DNA-fragment (Martineau et al., 1998), the mecA gene (De Neeling et al., 1998), and the Panton-Valentine leucocidin toxin genes (Lina et al., 1999).

2.2. Typing of MRSA

MRSA-isolates were typed by PFGE using SmaI as restriction enzyme according to the Harmony protocol (Murchan et al., 2003). A sample of 104 MRSA isolates from pigs (1–3 per group) were typed by spa-typing (Harmsen et al., 2003) and 1 isolate per group was subjected to Multi-Locus Sequence Typing (MLST) (Enright et al., 2000). Typing of the Staphylococcal Chromosome Cassette (SCC meC) was performed by PCR (Zhang et al., 2005).

2.3. Susceptibility testing

The susceptibility to antimicrobials of one isolate per group was tested by agar dilution using Mueller Hinton Agar (BBL) and multipoint inoculation (Clinical and Laboratory Standards Institute, 2006). The antibiotics tested were clindamycin (Pharmacia), teicoplanin (Aventis Pharma), mupirocin (Glaxo-SmithKline), linezolid (Pfizer), chloramphenicol, ciprofloxacin, doxycycline, erythromycin, fusidic acid, gentamicin, kanamycin, neomycin, oxacillin, rifampicin, tetracycline, tobramycin, trimethoprim-sulfamethoxazole (co-trimoxazole) and vancomycin (MP Biomedicals). S. aureus ATCC 43300 and S. aureus ATCC 29213 were used as reference strains.

2.4. Statistical analysis

Statistical analyses were performed in GAUSS (Aptech Systems, Inc. Black Diamond, WA, USA).
A two level logistic-normal model was used, assuming a normal distribution both of the log(odds) among groups within slaughterhouses, and of the log(odds) among slaughterhouses. Fitting was by maximum likelihood and profile likelihood was used to obtain confidence bounds of the variance parameters.

3. Results

3.1. Prevalence of MRSA in pigs

MRSA was found in 209 (39%) of the 540 screened pigs. At least 1 of the 10 sampled pigs carried MRSA in 44 (81%) of the 54 investigated groups. The number of MRSA-carrying animals per group of 10 pigs is given in Table 1.

The log(odds) of MRSA carrying pigs differed significantly among groups (\( p < 0.0001 \)) and among slaughterhouses (\( p < 0.0001 \)). The normal logistic model showed a variance of the log(odds) among groups of 2.2 (95% CI 1.0–4.5), and an almost equally large variance among slaughterhouses of 2.1 (95% CI 0.65–7.5). The geographic location of the farmers who supplied the groups of pigs appeared representative for the distribution of pigs over The Netherlands. Groups with a high number of MRSA carrying pigs did not cluster regionally.

Swabs taken from the nares of pigs in the slaughterhouses were incubated in an enrichment broth containing mannitol and the pH indicator phenol red. The enrichment broth turned yellow in nearly all tubes during incubation indicating growth of mannitol fermenting organisms. However, the subsequent MRSA-selective agar media and the PCR showed only a minority of these bacteria to be MRSA. We did not systematically subculture or identify the other bacteria. Some were oxacillin resistant *Staphylococcus lentus*, *Staphylococcus sciuri* and *Enterococcus faecalis* and oxacillin sensitive *S. aureus*, *Staphylococcus chromogenes* and *Staphylococcus simulans*.

3.2. Molecular typing

The predominant *spa* types of the NT MRSA from the pigs were t011, t108 and t1254, whereas *spa* types t1255, t567, t034 and t943 were found sporadically (Table 2). All *spa* types were closely related. Type t1254 was found only in slaughterhouse 5, where 13 of the 14 selected MRSA in 5 of the 6 groups belonged to this *spa* type. *Spa* type t1254 differs by only 1 base substitution (G to C) in the first repeat from *spa* type t011.

All isolates showed ST 398. SCCmec types IVa (\( n = 41 \)) and V (\( n = 59 \)) were most prevalent, whereas type III was present in only four isolates. We did not detect the genes of Panton-Valentine Leucocidin in any isolate.

3.3. Susceptibility testing

The oxacillin MICs of the NT MRSA from pigs were relatively low (Table 3). All strains were intermediate or resistant to doxycycline and resistant to tetracycline. The 10 isolates (23%) resistant to erythromycin were cross-resistant to clindamycin.
All 16 strains (36%) resistant to kanamycin were cross-resistant to gentamicin and all but 1 were cross-resistant to tobramycin. Nearly all tested isolates were susceptible to ciprofloxacin, co-trimoxazole, rifampicin, teicoplanin, vancomycin, linezolid, amikacin, chloramphenicol, fusidic acid and mupirocin.

4. Discussion

We found an unexpected high prevalence of MRSA in healthy pigs originating from more than 50 different farms in The Netherlands. In our country the prevalence of MRSA in companion animals and horses is low. However, we detected MRSA in 39% of the 540 pigs, in 81% of the 54 groups of 10 pigs and in all 9 slaughterhouses. All of the MRSA isolated from the pigs were non-typeable by PFGE using SmaI macrorestriction. We conclude that NT MRSA has widely spread in the Dutch pig population. These results are in line with the earlier isolation of NT MRSA from pigs and humans on the same farm (Huijsdens et al., 2006).

However, it is likely that the number of positive groups was raised considerably by transmission of the NT MRSA in the lairages of the slaughterhouses. We found a significant difference in the prevalence of MRSA-positive pigs among slaughterhouses. Thirteen of 14 tested pigs delivered to slaughterhouse no. 5 had the same spa type, which differed from the main spa type t011 in one nucleotide. Two delivering farms did not obtain pigs from elsewhere and four had received pigs from several rearing farms. So the aberrant dominant spa type in slaughterhouse no. 5 was probably due to transmission of this particular MRSA strain among groups of pigs from different farms in that slaughterhouse.

The origin of the NT MRSA in the pigs remains unclear. In The Netherlands, there are three types of pig farms: breeding farms, rearing or reproduction farms and fattening farms. Farmers may rear piglets at the same farm or they buy piglets for fattening (finishing pigs) at rearing farms. To date, we do not know if the pigs get infected at the fattening farms or if they have already been infected when they arrive on these farms. If the pigs on the breeding farms or rearing farms are colonized with MRSA, finishing pigs will be contaminated too.

Possible sources of the mecA gene are coagulase negative staphylococci belonging to the normal microflora of the pig, which may have transmitted the mecA gene to a methicillin susceptible S. aureus strain. Alternatively, the MRSA strain as such may have been transmitted to pigs from another source. Perhaps pigs are just a good host for this special MRSA strain, which originated from another host or possibly feed. In household contacts humans may infect companion animals with MRSA and vice versa. A similar phenomenon may have been the initial cause of the emergence of the NT MRSA in pigs.

MRSA may also be disseminated from contaminated feed and dust. Tetracycline resistant S. aureus might survive or even thrive in feed medicated with tetracyclines. Gibbs et al. (2004) detected resistant S. aureus inside and downwind of swine confinement facilities in levels, which they considered a potential

<table>
<thead>
<tr>
<th>Spa type</th>
<th>Tandem repeats</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>t011</td>
<td>008-16-02-25---34-24-25</td>
<td>43</td>
</tr>
<tr>
<td>t108</td>
<td>008-16-02-25---24-25</td>
<td>39</td>
</tr>
<tr>
<td>t1254</td>
<td>106-16-02-25---34-24-25</td>
<td>13</td>
</tr>
<tr>
<td>t1255</td>
<td>008-16-24-25</td>
<td>4</td>
</tr>
<tr>
<td>t567</td>
<td>008-----02-25---24-25</td>
<td>3</td>
</tr>
<tr>
<td>t943</td>
<td>008-16-02-25-25---24-25</td>
<td>1</td>
</tr>
<tr>
<td>t034</td>
<td>008-16-02-25-02-25-34-24-25</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>104</td>
</tr>
</tbody>
</table>
human health hazard. They found *S. aureus* to be the predominant bacterium in the air within a swine barn, being present at $10^4$ CFU/m$^3$, and concluded that swine facilities should be placed at least 200 m from residential areas to avoid detrimental effects on human health (Green et al., 2006). According to these authors, pig farmers should wear particle respirators and should change clothes and shower prior to leaving the barn to prevent exposure of vulnerable populations (children, elderly, immunocompromised individuals) to bacteria from the swine barn adhering to their clothes and skin.

The significant difference in the prevalence of MRSA-positive pigs among groups may have been due to transmission among pigs within groups and to differences in risk factors among the farms or farm

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>≤0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>≥128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>24</td>
<td>15</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>42</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>21</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>22</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacine</td>
<td>43</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>14</td>
<td>12</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td>40</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linezolid</td>
<td>-</td>
<td>38</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>-</td>
<td>22</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Neomycin*</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amikacin</td>
<td>43</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>6</td>
<td>37</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fusidic acid*</td>
<td>24</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mupirocin*</td>
<td>44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) no growth; (*) no CLSI breakpoints; vertical lines are breakpoints.
compartment where the pigs were raised, particularly the use of tetracyclines which may select bacteria which are resistant to tetracycline. In 2004, the use of tetracyclines as group medication in breeding and fattening facilities for pigs was 10 resp. 9 daily doses per animal year, which may have been an underestimation (VANTURES, 2005). Other antibiotics were used in at least 10-fold lower amounts. In contrast sows and piglets in breeding facilities received much more penicillins and aminoglycosides as compared to pigs for fattening. So the resistance to the former antibiotics may have been due to selection in breeding facilities.

The selection effect of the considerable and predominant use of tetracyclines in pigs is supported by the finding that nearly all MRSA isolates from pigs were susceptible or intermediate to ciprofloxacin, co-trimoxazole and several other antibiotics. Resistance to erythromycin, clindamycin and the aminoglycosides kanamycin, gentamicin, tobramycin and neomycin was around 30%. Gentamicin MICs of the resistant strains were higher than the MICs of tobramycin and no correlation with neomycin-resistance was observed. These observations suggest the presence of the aac(6')-aph(2') aminoglycoside-modifying enzyme (Vanhoof et al., 1994; Ida et al., 2001). The gene for this enzyme might be present on SCCmec type IVa because all kanamycin resistant strains contained SCCmec type IVa, whereas the strains, which were susceptible or intermediate to kanamycin had SCCmec type III or V.

Our survey in pigs and the earlier isolation of NT MRSA from humans (Voss et al., 2005) indicate that MRSA could be much more frequent among persons having contact with pigs than among other persons outside hospitals (Wertheim et al., 2004). Persons at risk include pig farmers, transporters of pigs, personnel of slaughterhouses and veterinarians. A higher prevalence of S. aureus carrierism among pig farmers was noted earlier in France and 5 of 50 S. aureus isolates in that study were resistant to methicillin. These 5 MRSA isolates showed 4 different MLSTs and only 1 had ST 398 (Armand-Lefevre et al., 2005). In contrast to the French findings, our MRSA isolates from pigs all had ST 398. All the NT MRSA from pigs may have descended recently from a common ancestor by deletions or insertions of one or more repeats as shown by the alignment of their spa types (Table 2). In contrast to our findings in pigs, MRSA from companion animals in The Netherlands are typeable by the standard PFGE method and show a wide variety of different PFGE-, spa- and MLST types which are also common in humans (Van Duijkeren et al., 2006).

In conclusion, we observed a high prevalence of MRSA in pigs in Dutch slaughterhouses. Further research into causes and effects is needed.

Acknowledgements

We thank M.E.O.C. Heck, G.N. Pluister and L. de Heer for PFGE analyses and Dr. P.J. van der Wolf and Ir. H. Rang for their comments on the manuscript.

References


