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## High Prevalence of Methicillin Resistant in Pigs

A.J. de Neeling, M.J.M. van den Broek, E.C. Spalburg, M.G. van Santen-Verheuveel, W.D.C. Dam-Deisz, H.C. Boshuizen, A.W. van de Giessen, E. van Duijkeren, X.W. Huijsdens

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1 High Prevalence of Methicillin Resistant *Staphylococcus aureus* in Pigs

2

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**18 Abstract**

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

25 All MRSA isolates belonged to 1 clonal group, showing Multi-Locus Sequence Type  
26 398 and closely related *spa* types (mainly t011, t108 and t1254). Three types of the  
27 Staphylococcal Chromosome Cassette (*SCCmec*) were found: III (3%), IVa (39%) and V  
28 (57%). All 44 tested isolates (1 isolate per group) were resistant to tetracycline, reflecting the  
29 high and predominant use of tetracyclines in pig husbandry. Twenty-three percent of the  
30 isolates were resistant to both erythromycin and clindamycin and 36% to kanamycin,  
31 gentamicin and tobramycin but only a single isolate was resistant to co-trimoxazole and none  
32 to ciprofloxacin and several other antibiotics.

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

**38 Keywords**

39 Swine microbiology, *Staphylococcus aureus*, methicillin resistance, tetracycline resistance, genetics,  
40 Netherlands

41

## 42 **Introduction**

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

49 Recently Voss et al. (2005) isolated MRSA from 3 patients who had contact with  
50 pigs. These authors also tested 26 pig farmers. Six of them (23%) carried MRSA.  
51 Subsequently MRSA was isolated from several members of a family living on a pig farm and  
52 8 out of 10 pigs at the same farm carried MRSA (Huijsdens et al., 2006). All MRSA-isolates  
53 from human and porcine origin in these investigations were non-typeable by standard  
54 Pulsed-Field Gel Electrophoresis (PFGE) using the *SmaI* restriction enzyme (NT). The NT  
55 MRSA contain a restriction modification enzyme which methylates the *SmaI*-recognition  
56 sequence (Bens et al., 2006).

57 These observations prompted us to determine the prevalence of MRSA in healthy  
58 pigs in 9 Dutch slaughterhouses. We further analyzed the porcine MRSA by molecular  
59 typing and susceptibility testing.

60

## 61 **Materials and Methods**

62

### 63 **Survey in nine slaughterhouses**

64 From November 2005 to January 2006 in each of 9 slaughterhouses all over the  
65 Netherlands 6 groups, 10 pigs per group, 540 pigs in total, were screened. In 2005 63% of  
66 the pigs raised in the Netherlands were slaughtered in the nine investigated slaughterhouses.

67 In the Netherlands each slaughterhouse buys pigs from a broad range of farms, with few  
68 exclusive contracts between a slaughterhouse and the farms supplying pigs. So we are  
69 confident that we have screened a representative sample of the pigs in the Dutch  
70 slaughterlines. A group consisted of 10 consecutive pigs in the slaughterline, each group  
71 from a different farm, except 1 group which was composed of pigs from 3 farms and 2  
72 groups which were both from 1 farm.

73 A swab (Medical Wire & Equipment Co. (Bath) Ltd. Corsham, Wiltshire, no.  
74 MW102) was taken from the nares of the pigs just after stunning, by officials of the Dutch  
75 Food and Consumer Product Safety Authority (VWA). Within 5 hours after sampling, swabs  
76 were transferred into tubes containing 5 ml Phenol Red Mannitol Broth (Brunschwig  
77 Chemie, Amsterdam) with 4 mg/L oxacillin (Sigma) and 75 mg/L aztreonam (ICN). After 18  
78 h incubation at 35°C, the bacteria from each tube were plated onto sheep blood agar and 3  
79 selective agar media: MRSA Select Agar (BioRad, Veenendaal), Oxacillin Resistance  
80 Screening Agar and Chromogenic MRSA Agar (Oxoid, Haarlem).

81 After 18 h incubation at 35°C, suspected colonies were plated onto sheep blood agar  
82 and incubated for 18 h. Colonies suspect of being MRSA were tested by PCR for the *S.*  
83 *aureus* specific DNA-fragment (Martineau et al., 1998), the *mecA* gene (De Neeling et al.,  
84 1998), and the Panton-Valentine Leucocidin toxin genes (Lina et al., 1999).

85

### 86 **Typing of MRSA**

87 MRSA-isolates were typed by PFGE using *Sma*I as restriction enzyme according to  
88 the Harmony protocol (Murchan et al., 2003). A sample of 104 MRSA isolates from pigs (1  
89 to 3 per group) were typed by *spa*-typing (Harmsen et al., 2003) and 1 isolate per group was  
90 subjected to Multi-Locus Sequence Typing (MLST) (Enright et al., 2000). Typing of the

91 Staphylococcal Chromosome Cassette (SCC<sub>mec</sub>) was performed by PCR (Zhang et al.,  
92 2005).

93

#### 94 **Susceptibility testing**

95 The susceptibility to antimicrobials of 1 isolate per group was tested by agar dilution  
96 using Mueller Hinton Agar (BBL) and multipoint inoculation (Clinical and Laboratory  
97 Standards Institute, 2006). The antibiotics tested were clindamycin (Pharmacia), teicoplanin  
98 (Aventis Pharma), mupirocin (GlaxoSmithKline), linezolid (Pfizer), chloramphenicol,  
99 ciprofloxacin, doxycycline, erythromycin, fusidic acid, gentamicin, kanamycin, neomycin,  
100 oxacillin, rifampicin, tetracycline, tobramycin, trimethoprim-sulfamethoxazole (co-  
101 trimoxazole), and vancomycin (MP Biomedicals). *S. aureus* ATCC 43300 and *S. aureus*  
102 ATCC 29213 were used as reference strains.

103

#### 104 **Statistical analysis**

105 Statistical analyses were performed in GAUSS (Aptech Systems, Inc. Black  
106 Diamond, WA, USA). A 2 level logistic-normal model was used, assuming a normal  
107 distribution both of the log(odds) among groups within slaughterhouses, and of the log(odds)  
108 among slaughterhouses. Fitting was by maximum likelihood and profile likelihood was used  
109 to obtain confidence bounds of the variance parameters.

110

#### 111 **Results**

112

#### 113 **Prevalence of MRSA in pigs**

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

117 The log(odds) of MRSA carrying pigs differed significantly among groups ( $p <$   
118  $0.0001$ ) and among slaughterhouses ( $p < 0.0001$ ). The normal logistic model showed a  
119 variance of the log(odds) among groups of 2.2 (95% CI 1.0 to 4.5), and an almost equally  
120 large variance among slaughterhouses of 2.1 (95% CI 0.65 to 7.5). The geographic location  
121 of the farmers who supplied the groups of pigs appeared representative for the distribution of  
122 pigs over the Netherlands. Groups with a high number of MRSA carrying pigs did not cluster  
123 regionally.

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

132

### 133 **Molecular typing**

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



139 All isolates showed ST 398. SCC $mec$  types IVa (n=41) and V (n=59) were most  
140 prevalent, whereas type III was present in only 4 isolates. We did not detect the genes of  
141 Panton-Valentine Leucocidin in any isolate.

142

### 143 **Susceptibility testing**

144 The oxacillin MICs of the NT MRSA from pigs were relatively low (table 3). All  
145 strains were intermediate or resistant to doxycycline and resistant to tetracycline. The 10  
146 isolates (23%) resistant to erythromycin were cross-resistant to clindamycin.

147 All 16 strains (36%) resistant to kanamycin were cross-resistant to gentamicin and all  
148 but 1 were cross-resistant to tobramycin. Nearly all tested isolates were susceptible to  
149 ciprofloxacin, co-trimoxazole, rifampicin, teicoplanin, vancomycin, linezolid, amikacin,  
150 chloramphenicol, fusidic acid and mupirocin.

151

### 152 **Discussion**

153 We found an unexpected high prevalence of MRSA in healthy pigs originating from  
154 more than 50 different farms in the Netherlands. In our country the prevalence of MRSA in  
155 companion animals and horses is low. However, we detected MRSA in 39% of the 540 pigs,  
156 in 81% of the 54 groups of 10 pigs and in all 9 slaughterhouses. All of the MRSA isolated  
157 from the pigs were non-typeable by PFGE using *Sma*I macrorestriction. We conclude that  
158 NT MRSA has widely spread in the Dutch pig population. These results are in line with the  
159 earlier isolation of NT MRSA from pigs and humans on the same farm (Huijsdens et al.,  
160 2006).

161 However, it is likely that the number of positive groups was raised considerably by  
162 transmission of the NT MRSA in the lairages of the slaughterhouses. We found a significant  
163 difference in the prevalence of MRSA-positive pigs among slaughterhouses. Thirteen of 14

164 tested pigs delivered to slaughterhouse no. 5 had the same *spa* type which differed from the  
165 main *spa* type t011 in one nucleotide. Two delivering farms did not obtain pigs from  
166 elsewhere and four had received pigs from several rearing farms. So the aberrant dominant  
167 *spa* type in slaughterhouse no. 5 was probably due to transmission of this particular MRSA  
168 strain among groups of pigs from different farms in that slaughterhouse.

169         The origin of the NT MRSA in the pigs remains unclear. In the Netherlands, there are  
170 3 types of pig farms: breeding farms, rearing or reproduction farms and fattening farms.  
171 Farmers may rear piglets at the same farm or they buy piglets for fattening (finishing pigs) at  
172 rearing farms. To date, we do not know if the pigs get infected at the fattening farms or if  
173 they have already been infected when they arrive on these farms. If the pigs on the breeding  
174 farms or rearing farms are colonized with MRSA, finishing pigs will be contaminated too.

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

182         MRSA may also be disseminated from contaminated feed and dust. Tetracycline  
183 resistant *S. aureus* might survive or even thrive in feed medicated with tetracyclines. Gibbs  
184 et al. (2004) detected resistant *S. aureus* inside and downwind of swine confinement facilities  
185 in levels which they considered a potential human health hazard. They found *S. aureus* to be  
186 the predominant bacterium in the air within a swine barn, being present at  $10^4$  CFU/m<sup>3</sup>, and  
187 concluded that swine facilities should be placed at least 200 m from residential areas to avoid  
188 detrimental effects on human health (Green et al., 2006). According to these authors, pig

189 farmers should wear particle respirators and should change clothes and shower prior to  
190 leaving the barn to prevent exposure of vulnerable populations (children, elderly,  
191 immunocompromized individuals) to bacteria from the swine barn adhering to their clothes  
192 and skin.

193         The significant difference in the prevalence of MRSA-positive pigs among groups  
194 may have been due to transmission among pigs within groups and to differences in risk  
195 factors among the farms or farm compartments where the pigs were raised, particularly the  
196 use of tetracyclines which may select bacteria which are resistant to tetracycline. In 2004 the  
197 use of tetracyclines as group medication in breeding and fattening facilities for pigs was 10  
198 resp. 9 Daily Doses per animal year, which may have been an underestimation (MARAN-  
199 2004). Other antibiotics were used in at least 10-fold lower amounts. In contrast sows and  
200 piglets in breeding facilities received much more penicillins and aminoglycosides as  
201 compared to pigs for fattening. So the resistance to the former antibiotics may have been due  
202 to selection in breeding facilities.

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

214 Our survey in pigs and the earlier isolation of NT MRSA from humans (Voss et al.,  
215 2005) indicate that MRSA could be much more frequent among persons having contact with  
216 pigs than among other persons outside hospitals (Wertheim et al., 2004). Persons at risk  
217 include pig farmers, transporters of pigs, personnel of slaughterhouses and veterinarians. A  
218 higher prevalence of *S. aureus* carriage among pig farmers was noted earlier in France and  
219 5 of 50 *S. aureus* isolates in that study were resistant to methicillin. These 5 MRSA isolates  
220 showed 4 different MLSTs and only 1 had ST 398 (Armand-Lefevre et al., 2005). In contrast  
221 to the French findings, our MRSA isolates from pigs all had ST 398. All the NT MRSA from  
222 pigs may have descended recently from a common ancestor by deletions or insertions of 1 or  
223 more repeats as shown by the alignment of their *spa* types (table 2). In contrast to our  
224 findings in pigs, MRSA from companion animals in the Netherlands are typeable by the  
225 standard PFGE method and show a wide variety of different PFGE-, *spa*- and MLST types  
226 which are also common in humans (Van Duinkerken et al., 2006).

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

229

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233

234

## References

235

- 236 Armand-Lefevre, L., Ruimy, R., Andremont, A., 2005. Clonal comparison of *Staphylococcus aureus*  
237 isolates from healthy pig farmers, human controls, and pigs. *Emerg. Infect. Dis.* 11, 711-714.
- 238 Bens, C.C., Voss, A., Klaassen, C.H., 2006. Presence of a novel DNA methylation enzyme in  
239 methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to  
240 uninterpretable results in standard pulsed-field gel electrophoresis analysis. *J. Clin.*  
241 *Microbiol.* 44, 1875-1876.
- 242 Busscher, J.F., van Duijkeren, E., Sloet van Oldruitenborgh-Oosterbaan MM, 2006. The prevalence  
243 of methicillin-resistant staphylococci in healthy horses in the Netherlands. *Vet. Microbiol.*  
244 113, 131-136.
- 245 Clinical and Laboratory Standards Institute, 2006. Methods for dilution antimicrobial susceptibility  
246 tests for bacteria that grow aerobically 7th Ed. Document M7-A7.
- 247 De Neeling, A.J., van Leeuwen, W.J., Schouls, L.M., Schot, C.S., van Veen Rutgers, A., Beunders,  
248 A.J., Buiting, A.G., Hol, C., Ligtoet, E.E., Petit, P.L., Sabbe, L.J., van Griethuysen, A.J.,  
249 van Embden, J.D., 1998. Resistance of staphylococci in The Netherlands: surveillance by an  
250 electronic network during 1989-1995. *J. Antimicrob. Chemother.* 41, 93-101.
- 251 Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing  
252 for characterization of methicillin-resistant and methicillin-susceptible clones of  
253 *Staphylococcus aureus*. *J. Clin. Microbiol.* 38, 1008-1015.
- 254 Gibbs, S.G., Green, C.F., Tarwater, P.M., Scarpino, P.V., 2004. Airborne antibiotic resistant and  
255 nonresistant bacteria and fungi recovered from two swine herd confined animal feeding  
256 operations. *J. Occup. Environ. Hyg.* 1, 699-706.
- 257 Green, C.F., Gibbs, S.G., Tarwater, P.M., Mota, L.C., Scarpino, P.V., 2006. Bacterial plume  
258 emanating from the air surrounding swine confinement operations. *J. Occup. Environ. Hyg.*  
259 3, 9-15.

- 260 Harmsen, D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D., Vogel, U., 2003. Typing  
261 of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel  
262 software for *spa* repeat determination and database management. *J. Clin. Microbiol.* 41,  
263 5442-5448.
- 264 Huijsdens, X.W., Van Dijke, B.J., Spalburg, E., van Santen-Verheuver, M.G., Heck, M.E.O.C.,  
265 Pluister, G.N., Voss, A., Wannet, W.J.B., De Neeling, A.J., 2006. Community-acquired  
266 MRSA and pig-farming. *Annals of Clinical Microbiology and Antimicrobials* 5, 26.
- 267 Ida, T., Okamoto, R., Shimauchi, C., Okubo, T., Kuga, A., Inoue, M., 2001. Identification of  
268 aminoglycoside-modifying enzymes by susceptibility testing: epidemiology of methicillin-  
269 resistant *Staphylococcus aureus* in Japan. *J. Clin. Microbiol.* 39, 3115-3121.
- 270 Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M.O., Gauduchon, V., Vandenesch, F.,  
271 Etienne, J., 1999. Involvement of Pantone-Valentine leukocidin-producing *Staphylococcus*  
272 *aureus* in primary skin infections and pneumonia. *Clin. Infect. Dis.* 29, 1128-1132.
- 273 MARAN-2004. Monitoring of antimicrobial resistance and antibiotic usage in animals in the  
274 Netherlands in 2004, p. 22.
- 275 Martineau, F., Picard, F.J., Roy, P.H., Ouellette, M., Bergeron, M.G., 1998. Species-specific and  
276 ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. *J. Clin.*  
277 *Microbiol.* 36, 618-623.
- 278 Murchan, S., Kaufmann, M.E., Deplano, A., de Ryck, R., Struelens, M., Zinn, C.E., Fusing, V.,  
279 Salmenlinna, S., Vuopio-Varkila, J., El Solh, N., Cuny, C., Witte, W., Tassios, P.T., Legakis,  
280 N., van Leeuwen, W., van Belkum, A., Vindel, A., Laconcha, I., Garaizar, J., Haeggman, S.,  
281 Olsson-Liljequist, B., Ransjo, U., Coombes, G., Cookson, B., 2003. Harmonization of  
282 pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-  
283 resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European  
284 laboratories and its application for tracing the spread of related strains. *J. Clin. Microbiol.* 41,  
285 1574-1585.
- 286 SWAB, 2006. NethMap 2006. Consumption of antimicrobial agents and antimicrobial resistance  
287 among medically important bacteria in the Netherlands.

- 288 Van Duijkeren, E., Jansen, M., Wannet, W.J.B., Heck, M.E., Fluit, A.C., 2006. Increasing prevalence  
289 of infections with methicillin-resistant *Staphylococci* in animals. Abstract. Ned. Tijdschr.  
290 Med. Micr. 14(suppl), S24.
- 291 Vanhoof, R., Godard, C., Content, J., Nyssen, H.J., Hannecart-Pokorni, E., 1994. Detection by  
292 polymerase chain reaction of genes encoding aminoglycoside-modifying enzymes in  
293 methicillin-resistant *Staphylococcus aureus* isolates of epidemic phage types. Belgian Study  
294 Group of Hospital Infections (GDEPIH/GOSPIZ). J. Med. Microbiol. 41, 282-290.
- 295 Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant *Staphylococcus*  
296 *aureus* in pig farming. Emerg. Infect. Dis. 11, 1965-1966.
- 297 Wertheim, H.F., Vos, M.C., Boelens, H.A., Voss, A., Vandenbroucke-Grauls, C.M., Meester, M.H.,  
298 Kluytmans, J.A., van Keulen, P.H., Verbrugh, H.A., 2004. Low prevalence of methicillin-  
299 resistant *Staphylococcus aureus* (MRSA) at hospital admission in the Netherlands: the value  
300 of search and destroy and restrictive antibiotic use. J. Hosp. Infect. 56, 321-325.
- 301 Zhang, K., McClure, J.A., Elsayed, S., Louie, T., Conly, J.M., 2005. Novel multiplex PCR assay for  
302 characterization and concomitant subtyping of staphylococcal cassette chromosome mec  
303 types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 43, 5026-  
304 5033.
- 305

306 Table 1. Number of NT MRSA carrying pigs in 6 groups of 10 pigs in each of 9  
 307 slaughterhouses.

Slaughterhouse no.	Date	Group						Total
		1	2	3	4	5	6	
1	08-11-05	7	5	5	0	1	1	19
2	14-11-05	0	1	1	1	2	0	5
3	21-11-05	0	0	2	0	2	1	5
4	28-11-05	0	0	8	7	9	2	26
5	05-12-05	5	8	9	1	4	1	28
6	12-12-05	5	2	6	7	6	4	30
7	19-12-05	10	10	8	6	6	10	50
8	09-01-06	6	1	8	10	9	4	38
9	16-01-06	1	4	1	0	2	0	8
Total								209

308



309 Table 2. *Spa* types of NT MRSA from pigs.

310

<i>Spa</i> type	Tandem repeats	Number
t011	008-16-02-25-----34-24-25	43
t108	008-16-02-25-----24-25	39
t1254	106-16-02-25-----34-24-25	13
t1255	008-16-----34-24-25	4
t567	008----02-25-----24-25	3
t943	008-16-02-25----25----24-25	1
t034	008-16-02-25-02-25-34-24-25	1
Total		104

311

312 Table 3. Minimum inhibitory concentrations of antibiotics (mg/L) for NT MRSA from pigs  
 313 (44 groups tested, 1 isolate per group). - = no growth; \*) no CLSI breakpoints. Vertical lines  
 314 are breakpoints.

Antibiotic	$\leq 0.25$	0.5	1	2	4	8	16	32	64	$\geq 128$
Oxacillin					-	4	24	15	1	-
Tetracycline			-	-	-	-	-	42	2	
Doxycycline			-	-	-	10	21	13		
Erythromycin	22	12	-	-	-	-	10			
Clindamycin	34	-	-	-	-	-	10			
Ciprofloxacin		43	-	1	-	-	-	-		
Co-trimoxazole	14	12	15	2	1	-				
Rifampicin	44	-	-	-	-	-				
Teicoplanin			44	-	-	-	-	-	-	
Vancomycin		1	40	3	-	-	-	-	-	
Linezolid		-	38	6	-	-	-	-	-	
Kanamycin			-	22	2	-	2	2	-	16
Neomycin*)			32	-	-	4	6	2		
Gentamicin			28	-	-	-	-	16		
Tobramycin			25	-	-	-	15	4		
Amikacin					43	1	-	-	-	
Chloramphenicol					6	37	-	1		
Fusidic acid*)	24	20	-	-	-	-				
Mupirocin*)			44	-	-	-	-	-	-	-

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