MICROBIAL DRUG RESISTANCE
Volume 18, Number 2, 2012

DOI: 10.1089/mdr.2011.0138

Prevalence and Antimicrobial Susceptibility of Methicillin-Resistant *Staphylococcus aureus* Among Pigs in Belgium

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The prevalence, distribution, and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) in Belgian pig farms has been investigated. To that end, nasal samples were collected from 1,500 pigs on 50 farms randomly selected over Belgium. Both closed (breeding or farrow-to-finish) and open (fattening) farms were included. Within closed farms different age groups were investigated. A total number of 663 (44%) pigs belonging to 34 (68%) farms carried MRSA. According to their management practice, MRSA was detected on 94% of the open farms and 56% of the closed farms. Focusing on the in-herd prevalence among fattening pigs for both management systems, a significantly higher rate was found in open farms (72%) compared to closed farms (26%). Within the closed farms, piglets (41%) showed a higher MRSA prevalence than sows (26%) and fattening pigs (26%). All strains tested were ST398 and showed mainly *spa*-type t011, as commonly found on pig herds in Europe. Less dominating *spa*-types were t034, t567, and t2970. The MRSA strains carried two SCC*mec*-types, type IVa or V. All 643 MRSA strains were resistant to tetracycline and additional resistances to trimethoprim (97%), lincosamides (73%), macrolides (56%), aminoglycosides (48%), and fluoroquinolones (32%) were found. Multiresistance (defined as resistance to four or more non-β-lactam antimicrobial classes) was found in 63% of the tested strains. In conclusion, a high prevalence of MRSA was found in Belgian pig farms, with the highest prevalence in open farms. In accordance with other European countries, age-related and management-related differences in MRSA prevalence were observed that should be considered when control strategies are outlined.

Introduction

During the last decade, an increasing number of studies reported the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in animals. In Europe, the strains found in livestock predominantly belong to multilocus sequence type 398 (ST398) and are commonly nontypeable (NT) by Pulsed-field gel electrophoresis (PFGE) using *Sma*I. Although MRSA ST398 was initially found associated with pigs in The Netherlands,[29] it has been shown that this clone globally prevails in a wide variety of animal species.[5,26] In addition, it has been demonstrated that colonized animals, predominantly pigs, act as a reservoir for MRSA ST398 carriage in humans.[6,20,22,23,34] In Belgium, 38% of the farm personnel has been reported carry MRSA ST398.[8] Fortunately, it was found that MRSA ST398 strains are primarily adapted to animals and do not easily spread among humans.[23,24,30] However, MRSA ST398 has repeatedly been found to cause infections in both animals and humans.[7,17,20,25,27,33] Given the observation that MRSA ST398 easily integrates DNA by horizontal gene transfer, the risk subsists that strains become more virulent

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and harder to treat.[1,14,15] Cases of Panton-Valentine leukocidin-positive MRSA ST398 have already been reported in The Netherlands,[22] Denmark,[17] Sweden,[32] Finland,[19] and China.[35]

Little information is available on the prevalence and distribution of MRSA on pig farms with different management practices. An European base-line survey in 2009 has reported that 40% of the Belgian breeding farms were MRSA-positive, with *spa*-type t011 as most dominant type.[10] However, in that baseline study, the pooling of environmental wipes probably resulted in substantial underestimation of the true prevalence, especially on farms with low in-herd prevalence.[4] Additionally, higher MRSA rates have been observed on farms downstream the pig production chain in The Netherlands.[3,28] Purchase of pigs from MRSA-positive herds has been identified as one of the risk factors for the emergence of MRSA on previously negative farms. Further, age-related differences in MRSA colonization have been reported.[9,31] Piglets might be more susceptible to MRSA colonization at weaning. Therefore, age and different management practices must be considered when comparing results of various surveillances and designing new control strategies.

This study aimed to (1) assess the prevalence and distribution of MRSA on different kinds of pig farms in Belgium, (2) determine antimicrobial susceptibility profiles of these MRSA isolates, and (3) characterize them by molecular typing (*spa*-typing, MLST and SCC*mec*-typing).

Materials and Methods

Sampling method

Fifty pig farms were randomly selected from the "Sanitel varken" database, provided by the Belgian Federal Agency for the Safety of the Food Chain (Fig. 1). The sampling was stratified by province and proportional to the number of active recorded herds. The selected farms were categorized as open ($n=34$) or closed ($n=16$). Open farms were defined as farms solely rearing fattening pigs, where the piglets came from different breeding farms. The closed farms were either breeding farms, or farrow-to-finish farms. Import of new animals into these farms was limited to absent. In the period between April 1, 2007 and August 1, 2007 the selected farms were contacted and visited by a veterinarian. Nasal swabs were collected from 30 healthy pigs per farm. When possible, the sampling was equally distributed over the different animal age groups (fattening pigs, sows, and piglets) present in the farm.

MRSA isolation and identification

Nasal samples ($n=1{,}500$) were transported in Stuart transport medium (Copan). Swabs were placed in enrichment broth medium made of brain heart infusion broth (Bio-Rad) supplemented with NaCl 7.5%. After 24 hrs incubation, broth was subcultured onto a selective chromogenic agar for MRSA (ChromID MRSA; BioMérieux) for 24 hrs. Suspected colonies were subcultured onto sheep blood agar. The identification

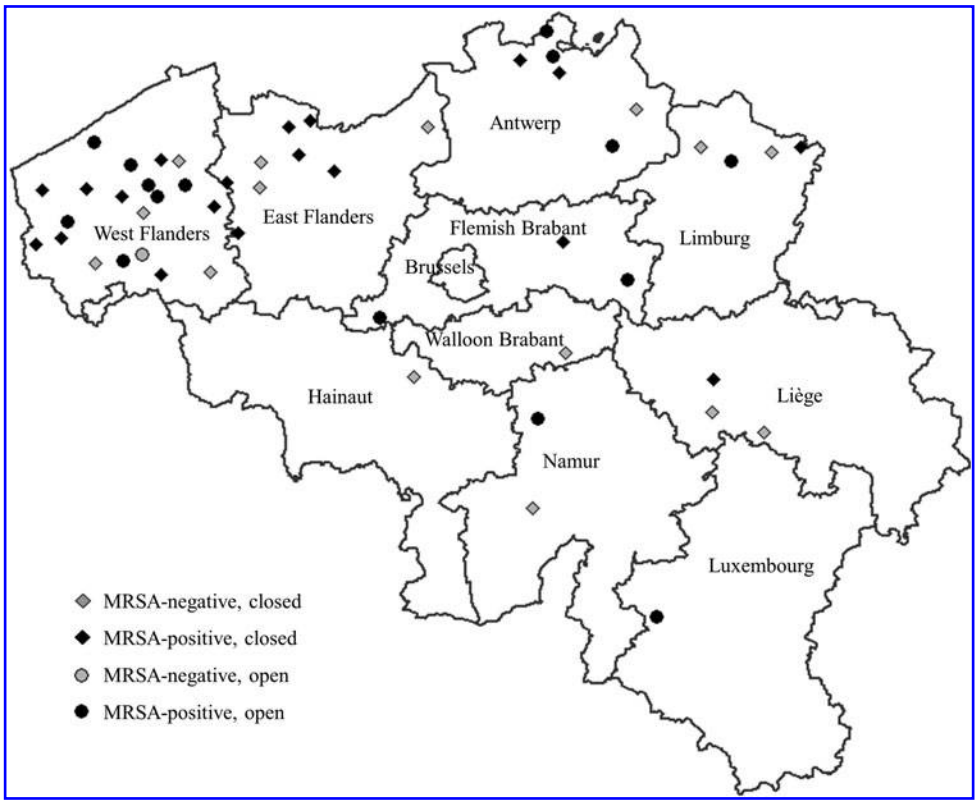

FIG. 1. Geographical distribution of the sampled farms ($n=50$). Distribution by methicillin-resistant *Staphylococcus aureus* (MRSA) status and farm type.

was then confirmed by 16S rRNA-*mecA*-*nuc* triplex polymerase chain reaction (PCR) as previously described.[18]

Antimicrobial susceptibility testing

For 643 confirmed MRSA strains, susceptibility to 16 non-β-lactam antimicrobial agents (tetracycline 80 μg, kanamycin 100 μg, gentamicin 40 μg, tobramycin 40 μg, trimethoprim 5.2 μg, sulfonamides 240 μg, erythromycin 78 μg, tylosin 150 μg, lincomycin 19 μg, pristinamycin 30 μg, ciprofloxacin 10 μg, chloramphenicol 60 μg, rifampicin 30 μg, fusidic acid 100 μg, mupirocin 10 μg, and linezolid 30 μg) was tested with the disk diffusion test using Rosco Neo-Sensitabs (Rosco Diagnostics) and following Rosco Diagnostics recommendations for inoculum preparation, inoculation, and incubation. Interpretation was done according to the information provided by Rosco Diagnostics (http://rosco.dk). The *Staphylococcus aureus* (*S. aureus*) strain ATCC 25923 was used as control strain.

Molecular characterization of the strains

In each MRSA-positive farm, a subset of strains was randomly selected for characterization by *spa*-typing,[12] that is, four in case of open farms and one per age group in case of closed farms ($n=113$). For closed farms with a MRSA-negative age group, one strain of another age group was selected. *Spa*-types were determined by using the Ridom Staph Type software version 1.3 (Ridom GmbH). Among these 113 strains, 15 were randomly selected for Multi-Locus Sequence Typing (MLST).[11] Five more were included for PFGE using *Sma*I as restriction enzyme.[13]

Determination of the Staphylococcal Cassette Chromosome mecsette (SCC*mec*) type was performed by multiplex PCR on the subset of strains characterized by *spa*-typing.[36]

Statistical analysis

A multivariate logistic regression was performed to identify risk factors for MRSA carriage in pigs, using STATA 9 SE. Statacorp 2005.[21] The dataset was recoded into categorical and dichotomic variables. The explanatory variables were either dichotomic (farm type: 0 = open, 1 = closed), or categorical (Province: by alphabetical order, and age group: 1 = piglets, 2 = fattening pigs, 3 = sows). The herd identification was treated as random effect to take into account the clustering effect of the chosen sampling procedure, that is, the fact that 30 samples have been collected in each sampled farm. A forward stepwise selection procedure of the explanatory variables and interactions was applied. Variables and interaction were maintained in the model when significant, with a p-value ≤ 0.05.

Results

Distribution and prevalence of MRSA in pigs

MRSA was detected in 34 out of the 50 farms (68%) sampled in the survey and in 663 (44%) of the 1,500 screened pigs (Fig. 1, Tables 1 and 2). A significant difference in the risk of infection with MRSA was observed between both farm management systems, showing a lower MRSA prevalence in closed farms (56%) than in open farms (94%) ($p<0.001$). Additionally, within the closed farms, the probability of being MRSA-positive significantly increased in the group of piglets (41%) compared with sows (26%) ($p=0.001$) and fattening pigs (26%) ($p=0.003$). However, when considering only the fattening pigs, significantly more fattening pigs carried MRSA in the open farms (72%) compared with the closed farms (26%). Location of the farms did not reveal significant geographic differences in the prevalence of MRSA.

Table 1. Number of Methicillin-Resistant *Staphylococcus aureus*-Positive Farms by Region, Province, and Farm Type

		Farm type		
		Open	*Closed*	*Total*
Region	*Province*	*No. MRSA + farms/ No. farms sampled*		
Flanders	Antwerp	3/3	2/3	5/6
	East Flanders	0/0	6/9	6/9
	Flemish Brabant	2/2	1/1	3/3
	Limburg	1/1	1/3	2/4
	West Flanders	7/8	8/12	15/20
	Total	13/14	18/28	31/42
Wallonia	Hainaut	0/0	0/1	0/1
	Liège	0/0	1/3	1/3
	Luxembourg	1/1	0/0	1/1
	Namur	1/1	0/1	1/2
	Walloon Brabant	0/0	0/1	0/1
	Total	2/2	1/6	3/8
Total Belgium		15/16	19/34	34/50

Antimicrobial resistance profile

All strains tested ($n=643$) were resistant to tetracycline (Fig. 2). Additional high resistance rates were found for trimethoprim ($n=623$, 97%), lincosamides ($n=472$, 73%), macrolides ($n=363$, 56%), aminoglycosides ($n=306$, 48%), and fluoroquinolones ($n=208$, 32%). Chloramphenicol resistance and sulfonamides resistance were low, 5% (30/643) and 2% (14/643), respectively. Only one strain was resistant to fusidic acid, another three to mupirocin, and one more to both antimicrobials. No resistance was found against linezolid and rifampicin (data not shown). Focusing on multiresistance, 63% ($n=405$) of the tested strains were resistant to at least four non-β-lactam antimicrobial classes.

The MRSA strains had 48 different resistance profiles, of which the four most prevalent profiles are given in Table 3.

Table 2. Number and Percentage of Methicillin-Resistant *Staphylococcus aureus*-positive Animals by Age Group and Farm Type

Production type	*No. MRSA-positive animals (%)*	*No. sampled animals*
Sows	88 (26)	340
Piglets	141 (41)	340
Fattening pigs (closed farms)	87 (26)	340
Fattening pigs (open farms)	347 (72)	480
Total	663 (44)	1,500

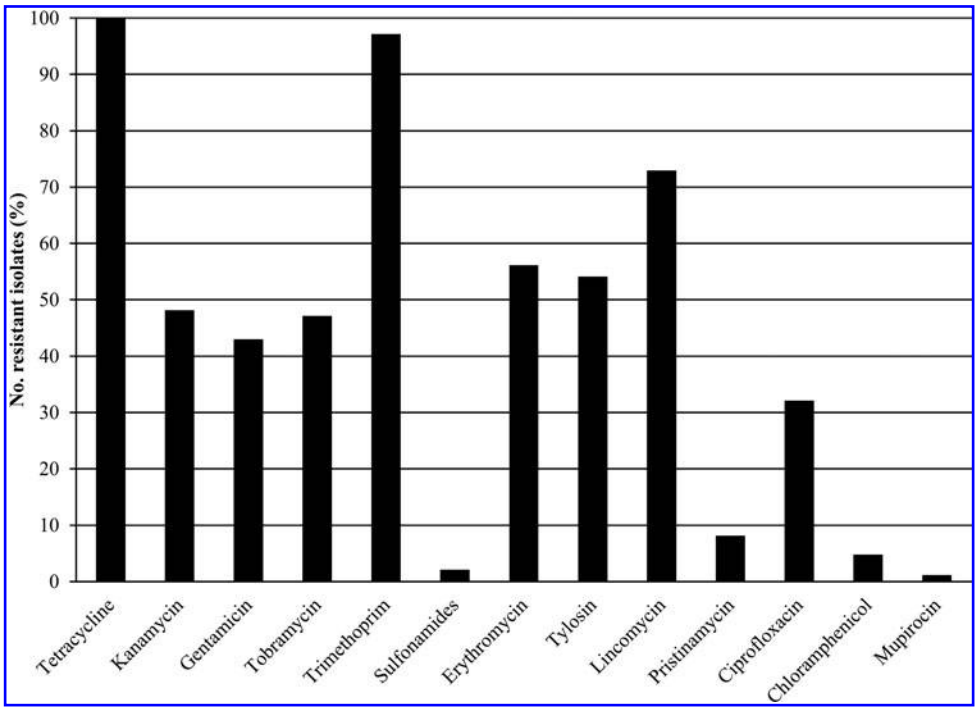

FIG. 2. Antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from pigs, Belgium ($n = 643$).

Only three farms had isolates with a single resistance profile; however, on two of these farms only one strain was isolated. On 10 farms (6 closed and 4 open) MRSA strains with two different resistance patterns were found. Seven farms (four closed and three open) showed three different resistance patterns. The other 14 farms showed four or more resistance patterns, with a maximum of 10 different patterns on one farm.

*Genetic background and SCC*mec*-type*

One hundred and five out of 113 strains tested were *spa*-types t011(Fig. 3). The other eight strains showed *spa*-type t034 (3/113), t2970 (3/113), and t567 (2/113). *Spa*-types t034 and t2970 were found, on one distinct farm, and *spa*-type t567 was found on two other farms. The 20 MRSA strains tested by PFGE were NT. The 15 strains (12 *spa*-type t011 and one of each less dominant *spa*-types) tested by MLST were ST398.

Table 3. Most Prevalent Antibiotic Resistance Profiles Found in Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from Pigs, Belgium

Resistance profile	*No. farms (%)*	*No. isolates (%)*
GEN-KAN-TOB-LIN-ERY-TYL-TET	14 (41)	163 (25)
CIP-TET-TMP	12 (35)	74 (11)
LIN-CIP-TET-TMP	11 (32)	78 (12)
TET-TMP	11 (32)	63 (10)

CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamycin; KAN, kanamycin; LIN, lincomycin; TYL, tylosin; TET, tetracycline; TMP, trimethoprim; TOB, tobramycin.

Most farms carried MRSA isolates with either SCC*mec*-type IVa (29% of the farms) or V (38% of the farms). In 11 farms (3 open and 8 closed farms), however, 2 different SCC*mec*-types were observed. Four strains (one *spa*-type t011, one t034, and two t567) harboring NT SCC*mec*-cassettes were isolated from three distinct farms.

Discussion

In this study, the MRSA prevalence and distribution among pigs in Belgium was evaluated by sampling 1,500 pigs among 50 farms. Forty-four percent of the pigs carried MRSA among 68% of the sampled farms, which is comparable with the prevalence of our neighboring countries.[10,28] Significantly higher numbers of MRSA-positive animals were found in open farms (94%) compared with closed farms (56%). Focusing on the prevalence within the fattening pigs, 72% of the animals housed in open farms were found MRSA-positive compared with 26% in closed farms. Regrouping of animals from multiple origins might be an explanation for the difference in prevalence between both management systems, since purchasing of animals from MRSA-positive suppliers has been described as a possible route of MRSA transmission within MRSA-negative herds.[3,28] Additionally, an age-related effect on MRSA-prevalence was identified in the closed farms with piglets presenting a higher risk for positive-samplings compared with sows or fattening pigs. Similar observations have already been described, which showed increased MRSA prevalence at weaning.[9,31] At that time, several factors such as stress, contact with MRSA-positive piglets, MRSA-positive weaning environment, human handling and antimicrobial use might result in increased risk for colonization.[9,31] A more in depth risk analysis is required to identify factors associated with age-related MRSA colonization.

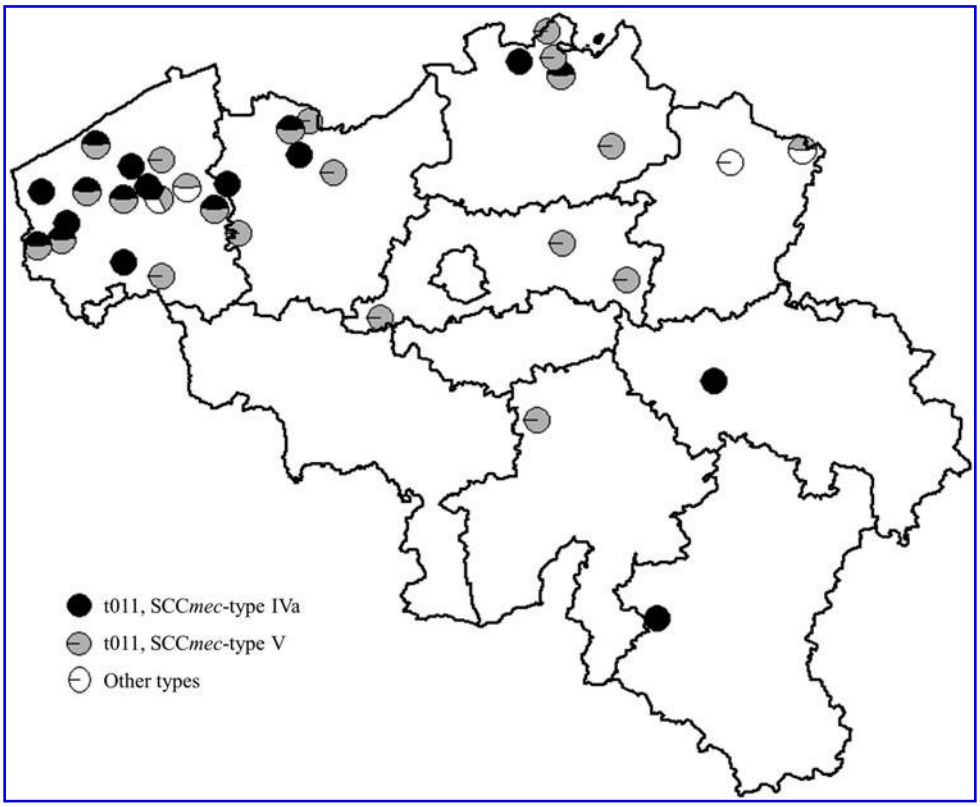

FIG. 3. Distribution, by farm, of methicillin resistant *Staphylococcus aureus* (MRSA) strains of *spa*-type t011-SCC*mec* type IVa, t011-SCC*mec* type V, and other types, Belgium. SCC, Staphylococcal Cassette Chromosome.

The genetic diversity of *spa*-types observed among the MRSA isolates was limited, possibly due to the small number of isolates selected for molecular typing. Within a same farm, however, MRSA isolates with the same *spa*-type can have different SCC*mec*-types. This indicates that the SCC*mec*-element has been introduced on multiple occasions in methicillin-susceptible *S. aureus* strains belonging to this specific genetic background,[28] and/or that importation of MRSA-positive pigs occurred more than once, from different sources, in those farms.

A high diversity in antimicrobial resistance profiles was found among the MRSA isolates analyzed in this study, which is consistent with data previously reported.[1,2,15,16] Besides β-lactam resistance, all strains were resistant to tetracycline, and nearly 100% of the tested strains showed trimethoprim resistance. In contrast to those high resistance rates, only 2% of the strains showed sulfonamides resistance, leaving the most in use combination trimethoprim-sulfonamides potentially therapeutically effective. Additional resistances to lincosamides (73%), macrolides (56%), aminoglycosides (48%) and fluoroquinolones (32%) were found. Fortunately, nearly no resistance against newer antibiotics, such as linezolid or MRSA-specific topical antibiotics was detected. However, neither of these is in use in veterinary medicine, meaning that few therapeutic options remain available for the treatment of infections caused by these strains in pigs and recommending susceptibility testing before starting a therapy. Consequently, it is advised to survey the evolution of resistance in MRSA ST398. This clone has been reported in people living or working with animals from 8 out of 15 European countries.[24] Moreover, several human infection cases have been demonstrated, even in persons without animal contact.[17,19,20,32]

In conclusion, this study confirms that MRSA is highly prevalent among Belgian pigs. Age-related and management-related differences in MRSA prevalence were observed, which are in accordance with other European countries. The high multiresistance rate once more highlights the importance of continual survey of the evolution of resistance in MRSA ST398 strains.

Acknowledgments

We thank Danielle Vandergheynst, Véronique Collet, and Sylvianne Rottiers for their skilled technical assistance. Dimitri Arijs and Frans Castrijck are acknowledged for sample taking. Stien Vandendriessche is acknowledged for her aid in creating the figures. We regret the death of Paul Heylen who helped preparing the sample taking.This study was financed by the Sanitary Fund of Belgium and likewise we thank all pig farmers of Belgium. We specifically thank the participating farmers who allowed us on their farms. The study was under supervision of the Belgian Antibiotic Policy Coordinating Committee (Bapcoc).

Funding for this study was provided by the Sanitary Fund of Belgium.

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