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MRSA CC398 in the pig production chain

E.M. Broens[a,b,*], E.A.M. Graat[a], P.J. van der Wolf[c], A.W. van de Giessen[b], E. van Duijkeren[d], J.A. Wagenaar[d,f], A. van Nes[e], D.J. Mevius[d,f], M.C.M. de Jong[a]

[a] Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[b] Centre for Infectious Disease Control Netherlands, National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[c] Pig Health Department, Animal Health Service, P.O. Box 9, 7400 AA Deventer, The Netherlands
[d] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.165, 3508 TD Utrecht, The Netherlands
[e] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.151, 3508 TD Utrecht, The Netherlands
[f] Central Veterinary Institute, P.O. Box 65, 8200 AB Lelystad, The Netherlands

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ABSTRACT

In 2005, a distinct clone of methicillin resistant *Staphylococcus aureus* (MRSA CC398) was found in pigs and people in contact with pigs. The structure of the pig production chain in high technology pig husbandry enables pathogens to spread during animal trading, with an increasing prevalence in herds further down the chain. The objective of this study was to quantify the effect of the MRSA status of the supplying herd on the MRSA status of the receiving herd in order to gain more insight into the role of animal trading as a transmission route for MRSA CC398. Nasal samples (60–80 pigs per herd) were collected from 38 herds; in 20 herds, environmental samples were collected as well. Ten MRSA-positive herds (based on the results of nasal swabs of 10 individual pigs per herd) from a prior study were included in the data analysis. Herds were classified as MRSA positive if at least one sample tested positive. The 48 herds were part of 14 complete (40 herds) and 4 incomplete (8 herds) pig production chains. Fifty-six percent of the herds were classified as MRSA positive. MRSA-positive herds were observed at the start (breeding herds), middle (farrowing herds) and the end (finishing herds) of the pig production chain. All of the herds in 8 chains tested MRSA positive;, all of the herds in 5 chains tested MRSA negative and in the remaining 5 chains, MRSA-positive and MRSA-negative herds were detected. Seven *spa* types were found, which were all previously confirmed to belong to CC398. All of the isolates were susceptible to mupirocin, linezolid, rifampicin, fusidic acid and cotrimoxazole. Resistance against tetracycline, erythromycin and clindamycin was found in 100, 74 and 76% of the isolates, respectively. Seventy-nine percent of herds with a MRSA-positive supplier of pigs were MRSA positive, whereas 23% of herds with a MRSA-negative supplier were MRSA positive (OR = 10.8; 95% CI: 1.5–110.1; $P = 0.011$). The presence of entirely MRSA-positive and MRSA-negative chains and the strong association between the MRSA status of herds and their suppliers illustrates a large risk associated with purchasing pigs from MRSA-positive herds; a top-down strategy for future control programs is, therefore, a basic requirement. However, 23% of herds with a MRSA-negative supplier were MRSA positive and furthermore, 46% of the herds at the top of the pig production chain without a supplier tested MRSA positive. This underlined the need for the identification of additional risk factors for MRSA.

* Corresponding author at: Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands. Tel.: +31 317 482921; fax: +31 317 485006.
E-mail address: els.broens@wur.nl (E.M. Broens).

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1. Introduction

In 2005, a distinct clone of methicillin resistant *Staphylococcus aureus* (MRSA CC398) was found in pigs and people in contact with pigs in The Netherlands (Voss et al., 2005). Since then, several other countries have detected MRSA CC398 in pig herds and other livestock (Graveland et al., 2008; Khanna et al., 2008; EFSA, 2009; Kock et al., 2009; Smith et al., 2009; Van Den Broek et al., 2009; Mulders et al., 2010). The prevalence of MRSA-positive pig herds varied from 23 to 70%, which might have been due to inconsistent sampling and laboratory techniques. Another explanation might be the selection of herds because risk factors for the introduction and persistence of MRSA on breeding herds might be completely different from risk factors for finishing herds. In the studies mentioned above, no explicit distinction was made between MRSA prevalence in different herd types, and reports on risk factors are limited. Van Duijkeren et al. (2008) showed in a pilot study, that 5 out of 6 herds supplying pigs to MRSA-positive herds were MRSA positive, which indicated transmission of MRSA within the pig production chain by the purchase of pigs. As breeding pigs constitute the top of the pig production chain, MRSA CC398 has the possibility to spread to a large number of farrowing herds by the trade of gilts and subsequently from these farrowing herds to an even larger number of finishing herds by the trade of piglets. The study objective was to quantify the effect of the MRSA status of the supplying herd on the MRSA status of the receiving herd to gain insight into the role of animal trade as a transmission route for MRSA CC398.

2. Materials and methods

2.1. Study design and sampling

In 2007 and 2008, 38 herds were sampled, including 11 breeding, 5 breeding-to-farrowing, 7 farrowing, 5 farrowing-to-finishing, and 10 finishing herds. Breeding herds were defined as herds with (pure) breeding pigs that supply gilts and/or boars to farrowing herds; no pigs enter these herds. Farrowing herds were defined as herds with farrowing pigs that supply pigs to finishing herds. Finishing herds were defined as herds that supply pigs for slaughter. Herds sometimes combined 2 of the previously mentioned disciplines, i.e., breeding and farrowing or farrowing and finishing. A pig production chain was defined as a number of pig farms linked to each other by the trade of animals. To enable reliable quantification of the effect of the MRSA status of the supplying herd on the MRSA status of the receiving herd, only herds with a maximum of 2 pig suppliers were selected. Herds within the same pig production chain were identified using the Dutch Identification and Registration System. Registration data were checked to make sure that farms did not change pig suppliers within a year before sampling. The time between sampling herds within a chain varied from several hours to 6 months. Nasal swabs (Medical Wire and Equipment, MW102, United Kingdom) were collected from either 60 or 80 pigs per herd. This sample size enabled MRSA to be detected in herds with a within-herd prevalence of 2–5%. Swabs were taken from each age group (sows, suckling piglets, weaners, finishers and rearing pigs) present in the herd. Additional samples, i.e., 5 environmental wipes (Sodibox, s1 kit ringer solution, France) were collected in 20 herds. Information on batch treatment with antimicrobials was available for 34 farms that participated in another study.

Data from 10 herds (2 breeding, 1 breeding-to-farrowing, 4 farrowing and 3 finishing herds) which were used by Van Duijkeren et al. (2008) in a previous study, were included in the analysis. In that previous study, 10 individual pigs per herd were sampled to determine the MRSA-status. Ten samples per herd enabled MRSA to be detected in herds with a within-herd prevalence of 25%. Therefore, only MRSA-positive herds found it that study were included in our study.

The 48 herds were part of 14 complete (40 herds) and 4 incomplete (8 herds) independent pig production chains. Only 1 herd was missing in each incomplete chain due to noncooperative farmers. Pig production chains consisted of at least 2 and at most 5 herds; two herds were used in the case of incomplete chains or when herds combining 2 disciplines were involved.

2.2. Laboratory analysis

All samples were sent to the Animal Health Service for analysis, which took place within 10 days after sampling. Analyses were performed on individual environmental wipes and pooled nasal swabs (4–6 swabs per pool) with each pool containing swabs from just one section of the herd and age group. First, samples were enriched using Mueller Hinton Broth with 6.5% NaCl (MHB+). Nasal swabs were placed into 10 ml MHB+ and environmental wipes were placed into 100 ml MHB+. After 18 h of aerobic incubation at 37 °C, 1 ml of MHB+ was transferred into 9 ml of Phenol Red Mannitol Broth with 75 mg/l aztreonam and 4 mg/l ceftizoxime (PMB+; BioMérieux, NL020, France). This selective enrichment broth was incubated aerobically for 18 h at 37 °C. A loop-full of PMB+ was spread onto sheep blood agar (Oxoid, PB5008A, United Kingdom) and a chromogenic MRSA screen agar (Oxoid, PO5196A, United Kingdom). One suspected colony per sample was confirmed by 2 PCR tests for the *S. aureus* specific DNA-fragment (De Neeling et al., 1998) and the *mecA* gene (Martineau et al., 1998). To confirm that MRSA isolates belonged to CC398 and to gain insight into the relatedness of isolates within a chain, isolates were typed by *spa* typing (Harmsen et al., 2003). Antimicrobial susceptibilities of at least 1 isolate per herd were determined quantitatively by broth microdilution with cation-adjusted Mueller-Hinton broth according to ISO standard 20776-1:2006. For broth microdilution, microtitre trays were used with custom-made panels of dehydrated dilution ranges of antibiotics (Sensititre®, Trek Diagnostic Systems, Basingstoke, UK). The ATCC strains *Enterococcus faecalis* 29212 and *Staphylococcus aureus* ATCC 29213 were included for quality control. The minimum inhibitory concentrations were defined as the lowest concentrations without visible growth. Breakpoints for classification of resistance and susceptibility were determined based on international and

Table 1
Breakpoints used for classification of resistance and susceptibility for different antimicrobials and the international standard in a study on MRSA CC398 in the pig production chain (The Netherlands, 2007/2008).

Antimicrobial	Breakpoint	International standard
Amikacin	$R \geq 32$	EUCAST
Ciprofloxacin	$R > 1$	EUCAST
Clindamycin	$R \geq 4$	CLSI M100-S17
Cotrimoxazole	$R \geq 4$	CLSI M100-S17
Erythromycin	$R \geq 8$	CLSI M100-S17
Fusidic acid	$R \geq 8$	CRG
Gentamicin	$R > 1$	EUCAST
Linezolid	$R > 4$	EUCAST
Mupirocin	$R > 4$	Not available
Neomycin	$R \geq 8$	Not available
Rifampicin	$R \geq 4$	CLSI M100-S17
Tetracycline	$R \geq 32$	CLSI M100-S17

EUCAST, European Committee on Antimicrobial Susceptibility Testing; CRG, Dutch Committee on guidelines on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute.

national standards (Table 1; www.eucast.org; CLSI, 2007). Human clinical breakpoints were primarily used to investigate resistance against antimicrobials used in human medicine. The antimicrobials tested in this study were amikacin, ciprofloxacin, clindamycin, cotrimoxazole, erythromycin, fusidic acid, gentamicin, linezolid, mupirocin, neomycin, rifampicin and tetracycline.

2.3. Data analysis

Herds were classified as MRSA positive if at least one sample (individual nasal sample, pool of nasal samples or environmental sample) tested positive. The association between the MRSA status of the receiving herds and the MRSA status of their supplier and application of batch treatments with antimicrobials were calculated using exact logistic regression analysis (SAS Institute Inc., 2004). The strength of association is presented in terms of odds ratios (OR). The estimated attributable fraction was calculated using the following equation: (OR-1)/OR (Noordhuizen et al., 2001).

Cohen's kappa, which is a measure of agreement between diagnostic methods, was calculated to compare the microbiological results of pooled animal samples and environmental samples (Cohen, 1960).

3. Results

Fifty-six percent (27/48; 95% CI: 41–71%) of the herds were classified as MRSA positive. MRSA-positive herds were observed in all types of herds within the pig production chain, ranging from 20.0% MRSA-positive farrowing-to-finishing herds (1 out of 5) to 100.0% MRSA-positive breeding-to-farrowing herds (6 out of 6) (Table 2). On average, 42% of the individual pigs tested MRSA positive in herds classified positive based on individual pig samples ($n = 10$). On average, 50% of the pooled samples and 20% of the environmental samples were MRSA positive in herds classified as positive based on pooled samples ($n = 7$) or on pooled and environmental samples ($n = 10$). In 3 herds, environmental wipes tested MRSA negative, even though the herd was classified as MRSA positive based on at least one MRSA-positive pool of nasal swabs. In the remaining 17 herds in which environmental samples were taken, the results of the environmental and pooled samples were consistent with the MRSA status of the herd; in 7 herds, environmental and pooled samples tested MRSA positive and in 10 herds, all of the samples tested MRSA negative. Cohen's kappa, a measure of agreement between the MRSA classification of a herd based on environmental wipes and pools of nasal swabs, was 0.70 (95% CI: 0.40–1.00).

All of the herds tested MRSA positive in 8 pig production chains, including 3 incomplete chains. All herds tested MRSA negative in 5 chains. MRSA-positive and MRSA-negative herds were observed in the remaining 5 chains, including 1 incomplete chain (Table 3). Sampling within a chain took place within 1 month in 11 chains. In the other 7 chains, the time intervals between sampling moments were between 1 and 6 months.

In 27 out of the 48 herds, the MRSA status of the supplier was known. The other 21 herds were breeding ($n = 13$) or breeding-to-farrowing ($n = 6$) herds without supplier or herds of which the supplier was not sampled (n = 2). Seventy-nine percent of the herds with a MRSA-positive supplier were MRSA positive, whereas 23% of herds with a MRSA-negative supplier were MRSA positive (OR = 10.8; 95% CI: 1.5–110.1; P = 0.011) (Table 4). The estimated attributable fraction was 0.91, which indicated that the MRSA status of 91% of the MRSA-positive herds was attributed to the MRSA-positive status of their supplier.

Antimicrobials were used as batch treatments on 74% (25/34) of the herds in which information on antimicrobial use was available. Herds that used antimicrobials as batch treatments were MRSA positive more often (76%) than herds that used antimicrobials incidentally (22%; OR = 10.2; 95% CI: 1.4–126.1; $P = 0.015$) (Table 4). Bivariable logistic regression with supplier status and batch treatment was possible for only 21 herds and showed no significant effect of batch treatments on MRSA status. Nine out of 14 herds (64%) that used batch treatments were MRSA positive, while only 2 out of 7 herds (30%) that did not use batch

Table 2
Total number of herds and number and percentage with exact 95% confidence interval of MRSA-positive herds in a study on MRSA CC398 in the pig production chain (The Netherlands, 2007/2008).

Herd type	*n* Total	*n* Positive	% Positive	95% CI
Breeding	13	6	46.2	19.2–74.9
Breeding-to-farrowing	6	6	100.0	54.1–100.0
Farrowing	11	5	45.5	16.8–76.6
Farrowing-to-finishing	5	1	20.0	0.5–71.6
Finishing	13	9	69.2	38.6–90.9
Total	48	27	56.3	41.2–70.5

Table 3
Total number and number of positive herds, samples per herd, herd type, pig supplier, MRSA status, *spa* type, resistance pattern and application of batch treatments in the pig production chains in a study on MRSA CC398 in the pig production chain (The Netherlands, 2007/2008).

Chain	Herd	Herd type	Pig supplier	MRSA status	Positive (total) number of			*Spa*-type (*n*)	Resistance pattern (*n*)	Batch treatment
					Pools	Wipes	Pigs			
Negative chains										
A[a]	1	a	–	neg	0 (20)					–
	2	c	1	neg	0 (15)					–
	3	e	2	neg	0 (10)	0 (5)				no
B[a]	4	a	–	neg	0 (20)					–
	5	a	–	neg	0 (20)					–
	6	d	4/5	neg	0 (10)	0 (5)				yes
C[a]	7	a	–	neg	0 (20)					–
	8	c	7	neg	0 (15)					–
	9	e	8	neg	0 (10)	0 (5)				no
D[a]	10	a	–	neg	0 (20)					–
	11	d	10	neg	0 (10)	0 (5)				yes
E	12	a	–	neg	0 (15)					no
	13	d	12	neg	0 (20)					yes
	14	d	12	neg	0 (20)					no
'Mixed' chains										
F	15	a	–	pos	1 (20)			t108 (1)	ClAENT (1)	–
	16	c	15	neg	0 (15)					–
	17	b	–	pos	14 (15)			t943 (13)	ClET (13)	–
								t2503 (1)	ClET (1)	
	18	e	16/17	pos	10 (10)			t108 (1)	ClET (1)	–
								t011 (1)	ClENT (1)	
								t943 (7)	ClET (7)	
								t2503 (1)	ClET (1)	
G[a]	19	a	–	pos	2 (20)			t108 (2)	ClET (2)	–
	20	c	19	neg	0 (10)	0 (5)				yes
	21	e	20	pos	2 (10)			t011 (2)	AGT (1)	no
									GT (1)	
H[a,b]	22	c	n.k.	neg	0 (10)	0 (5)				yes
	23	e	22	pos	1 (10)			t011 (1)	T (1)	–
I	24	a	–	pos	6 (10)	1 (5)		t011 (7)	GT (1)	yes
	25	c	24	pos	5 (10)	0 (5)		t108 (5)	ET (1)	yes
	26	e	25	neg	0 (10)	0 (5)				no
J	27	a	–	neg	0 (10)	0 (5)				no
	28	d	27	pos	5 (10)	1 (5)		t108 (6)	GT (1)	yes
	29	c	27	neg	0 (10)	0 (5)				yes
	30	e	29	neg	0 (10)	0 (5)				no
Positive chains										
K[a]	31	a	–	pos	4 (20)			t011 (3)	T (1)	yes
									ClEGNT (2)	
								t108 (1)	ClET (1)	
	32	c	31	pos			1 (10)	t108 (1)	ClET (1)	no
	33	e	32	pos			3 (10)	t108 (3)	ClET (3)	yes
L	34	b	–	pos			8 (10)	t108 (8)	ClET (8)	yes
	35	e	34	pos			2 (10)	t011 (1)	ClET (1)	yes
								t108 (1)	ClET (1)	

M[b]	36	c	n.k.	pos		8 (10)	t899 (7)	CIET (7)	yes	
							t1939 (1)	CIET(1)		
	37	e	36	pos		2 (10)	t899 (1)	T (2)	yes	
N[b]	38	a	–	pos		2 (10)	t108 (2)	ET (2)	yes	
	39	c	38	pos		3 (10)	t108 (3)	ET (3)	yes	
O[b]	40	a	–	pos		4 (10)	t567 (4)	T (2)	yes	
								CIT (2)		
	41	c	40	pos		9 (10)	t567 (9)	CIT (5)	yes	
								T (4)		
P	42	b	–	pos	1 (5)		t011 (6)	CiCIET (1)	yes	
							t108 (4)			
	43	e	42	pos	2 (5)		t108 (7)	CiCIET (1)	yes	
							t011 (1)			
Q	44	b	–	pos	1 (5)		t011 (8)	ACIEGT (1)	yes	
	45	b	–	pos	0 (5)		t011 (6)	CIET (1)	yes	
	46	e	44/45	pos	3 (5)		t011 (13)	CIEGNT (1)	yes	
R	47	b	–	pos	0 (5)		t011 (5)	NT (1)	yes	
	48	e	47	pos	1 (5)		t011 (10)	CIENT (1)	–	

a: breeding herd; b: breeding-to-farrowing herd; c: farrowing herd; d: farrowing-to-finishing herd; e: finishing herd n.k.: not known; A: amikacin; Ci: ciprofloxacin; Cl: clindamycin; E: erythromycin; G: gentamicin; N: neomycin; T: tetracyclin.

[a] Time between sampling 1–6 months.

[b] Incomplete chain (1 herd is missing).

treatments were MRSA positive (OR = 2.3; 95% CI: 0.1–48.0; $P = 0.836$). The effect of supplier status in the bivariable analysis (OR = 10.2; 95% CI: 1.1–156.8; $P = 0.042$) was consistent with the effect estimated in the univariable analysis on these 21 herds.

All of the MRSA isolates ($n = 154$) were *spa* typed (Tables 3 and 5). Seven *spa* types were identified, which were closely related and formerly confirmed to belong to CC398. *Spa* types t011 and t108 were detected most frequently and were identified in 42% and 28% of the isolates and in 48% and 52% of the positive herds, respectively. In 5 out of 27 positive herds, t011 and t108 occurred simultaneously within the herd. The less frequently identified *spa* types t567 (8%), t899 (6%), t943 (13%), t1939 (1%) and t2503 (1%), were found in herds within 1 pig production chain. *Spa* type t899 together with t1939 in chain M and *spa* type t943, t2503, t011 and t108 were found in chain F. *Spa* type t567 was only found in chain O. In 20 out of 27 MRSA-positive herds, only 1 *spa* type was found. In 6 herds, 2 different *spa* types were found, and in 1 herd, 4 different *spa* types were found (Table 3). In all of the cases where 2 spa types were found in 1 herd, there were 2 isolates that differed by only 1 repeat in the alignment of tandem repeats in the staphylococcal protein A region (Table 5).

Antimicrobial susceptibilities were determined for 86 MRSA isolates (Table 6). All of the isolates were susceptible to mupirocin, linezolid, rifampicin, fusidic acid and cotrimoxazole. Resistance against tetracycline, erythromycin and clindamycin was found frequently (100.0%, 74.4% and 75.6% of the isolates, respectively) and often in combination. Combined resistance against erythromycin and clindamycin was found in 67.4% (58/86) of the isolates. Resistance against aminoglycosides ranged from 3.5% for amikacin to 15.9% for neomycin. Resistance against ciprofloxacin was found in 2 isolates, *spa* types t011 and t108 in chain P. Multiresistance (resistance against >3 different antimicrobial classes) was found in 9.3% (8/86) of the isolates: 6 times in *spa* type t011 and 2 times in *spa* type t108.

4. Discussion

This study observed completely MRSA-negative and -positive pig production chains and an 11 times higher odds for herds with a MRSA-positive supplier to be MRSA-positive. These results confirm the hypothesis of Van Duijkeren et al. (2008) that animal trading was an important factor in the transmission of MRSA between pig herds. The occurrence of the same *spa* type, including some uncommon *spa* types and ciprofloxacin resistance within the same production chain supports the likelihood of MRSA transmission between pig herds by the trade of animals. However, the sample size was too small to statistically validate the findings on *spa* types and resistance patterns. The MRSA status of a vast majority of MRSA-positive herds was attributed to the MRSA-positive status of their supplier. However, this was estimated by univariable analysis, which assumes that the relationship is causal, and confounding is absent.

The 2 predominant *spa* types, t011 and t108, observed in this study have been found in high numbers in previ-

Table 4
Frequency (n and %), MRSA prevalence (%) and odds ratio (OR) for a herd to be MRSA positive with 95% confidence interval (CI), and exact *P*-value, resulting from univariable analysis for status of supplier and antimicrobial batch treatment in a study on MRSA CC398 in the pig production chain (The Netherlands, 2007/2008).

Variable	Category	Frequency	MRSA (%)	OR	95% CI	*P*-exact
Status supplier	Positive	14(51.9)	78.6	10.8	1.5–110.1	0.011
	Negative	13(48.2)	23.1	Ref		
Batch treatment	Yes	25(73.5)	76.0	10.2	1.4–126.1	0.015
	No	9(26.5)	22.2	Ref		

Table 5
Spa types and their repeat succession of 154 MRSA isolates: total number and percentage of all isolates, number and percentage of positive herds (out of 27 positive herds) and number and percentage of positive chains (out of 13 positive chains) in which the type was found in a study on MRSA CC398 in the pig production chain (The Netherlands, 2007/2008).

Spa type	Repeat succession[a]	Isolates		Positiveherds		Positivechains	
		n	%	*n*	%	*n*	%
t011	08-16-02-25-34-24-25	64	41.6	13	48.1	9	69.2
t108	08-16-02-25-24-25	45	29.2	14	51.9	8	61.5
t567	08-02-25-24-25	13	8.4	2	7.4	1	7.7
t899	07-16-23-02-34	9	5.8	2	7.4	1	7.7
t943	08-16-02-25-25-24-25	20	13.0	2	7.4	1	7.7
t2503	08-16-02-25-25-25-24-25	2	1.3	2	7.4	1	7.7
t1939	07-23-02-34	1	0.6	1	3.7	1	7.7

[a] www.spaserver.ridom.de.

ous Dutch studies (De Neeling et al., 2007; Van Duijkeren et al., 2008; Huijsdens et al., 2009). These 2 *spa* types are closely related and differ by only one repeat in the alignment of tandem repeats in the staphylococcal protein A region, which was similar to two other combinations that were found in our study (*spa* types t943 and t2503 and *spa* types t899 and t1939). Little is known about the incidence of mutations and, subsequently, conversion from one *spa* type to another. Therefore, the simultaneous occurrence of two closely related *spa* types within one chain or even one herd could be a result of (1) two independent introductions of MRSA or (2) one single introduction followed by a mutation. The finding in our study of three combinations of *spa* types differing by just one repeat, indicated that new *spa* types might have emerged through mutation, or more specifically, by a loss of repeats. As the staphylococcal protein A is just a small part of the whole organism, other typing techniques could be useful in investigating the relatedness of different *spa* types. Experiments in vitro and in vivo might help to estimate the incidence of mutations in the staphylococcal protein A region.

The good agreement observed between the classification of herds based on results of the pools of nasal swabs and the results of the environmental samples were consistent with a previous study (Broens et al., 2010). The detection of MRSA in environmental samples implied the possibility of indirect transmission of MRSA between pigs within a herd and between pigs and humans (Van Den Broek et al., 2009).

The antimicrobial resistance patterns found were similar to other studies on MRSA CC398 (De Neeling et al., 2007; Smith et al., 2009; Wagenaar and Van De Giessen, 2009). High prevalence of resistance against macrolides, lincosamides and tetracyclins has been observed in previous studies. Resistance against tetracycline, lincosamides and macrolides in MRSA CC398 appears to depend on the presence of the *tetM*-gene (tetracycline) and the *erm*-

Table 6
Number of isolates tested per *spa* type with the resistance percentage for 12 antimicrobials in a study on MRSA CC398 in the pig production chain (The Netherlands, 2007/2008).

Spa-type/antimicrobial	t011		t108		t567		t899		t943		t1939		t2503		Total	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Amikacin	15	13	26	0	13	0	9	0	20	0	1	0	2	0	86	4
Ciprofloxacin	15	7	26	4	13	0	9	0	20	0	1	0	2	0	86	2
Clindamycin	15	60	26	73	13	54	9	78	20	100	1	100	2	100	86	76
Cotrimoxazole	15	0	26	0	13	0	9	0	20	0	1	0	2	0	86	0
Erythromycin	15	60	26	96	13	0	9	78	20	100	1	100	2	100	86	74
Fusidic acid	14	0	8	0	–	–	–	–	20	0	–	–	2	0	44	0
Gentamicin	15	47	26	0	13	0	9	0	20	0	1	0	2	0	86	9
Linezolid	14	0	8	0	–	–	–	–	20	0	–	–	2	0	44	0
Mupirocin	14	0	8	0	–	–	–	–	20	0	–	–	2	0	44	0
Neomycin	14	43	8	13	–	–	–	–	20	0	–	–	2	0	44	16
Rifampicin	14	0	8	0	–	–	–	–	20	0	–	–	2	0	44	0
Tetracyclin	15	100	26	100	13	100	9	100	20	100	1	100	2	100	86	100

genes (lincosamides and macrolides) (Witte et al., 2007). Resistance against ciprofloxacin appears to be rare in pig isolates, in contrast to poultry and veal isolates (Mulders et al., 2010; Wagenaar and Van De Giessen, 2009). In Dutch poultry and veal husbandry, batch treatment with quinolones is frequently applied, whereas in Dutch pigs, the use of quinolones is uncommon (MARAN, 2008). The use of quinolones was not reported in either of the herds in which a ciprofloxacin-resistant isolate was identified. Resistance against mupirocin, linezolid, rifampicin and fusidic acid was not identified, which is important because these antimicrobials are considered important to the control of MRSA in human medicine.

The presence of completely MRSA-negative chains and the strong association between the MRSA status of herds and their suppliers suggested that a top-down strategy should be a prerequisite for future control programs. Such programs are based on the principle of top-down eradication, which ensures the absence of the 'disease' from the entire pig production chain as described for *Salmonella* in broiler chickens in Denmark (Wegener et al., 2003). However, 46% of the self-supplying herds and 23% of the herds with a MRSA-negative supplier tested MRSA positive. Therefore, more research is needed to elucidate additional risk factors for the introduction and persistence of MRSA in pig herds.

'Mixed' chains were observed in addition to completely positive or negative chains. In some cases, the period between sampling of the herds within one chain was very long (up to 6 months), which might explain some of the 'mixed' chains. The MRSA status of the herd could have changed within this period. In addition, there were chains in which information on one herd was missing, because the farmer was not willing to cooperate.

Batch treatments with antimicrobials resulted in a higher prevalence in herds that were subjected to batch treatments compared herds that were not subjected to batch treatments. However, in multivariable analysis on a smaller number of herds, due to incomplete information, the effect of batch treatment was smaller and not significant. A multivariable analysis on a large number of herds is needed to identify and quantify the effect of antimicrobial use on MRSA.

5. Conclusion

The results of this study illustrated that the MRSA status of a pig supplier highly affects the MRSA status of the receiving herd. A top-down control strategy for MRSA is therefore a basic requirement in the pig production chain. However, additional risk factors for MRSA need to be identified because not all MRSA-positive herds could be attributed to pigs received from MRSA-positive herds.

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