Polish Journal of Microbiology
2017, Vol. 66, No 3, 383–392

ORIGINAL PAPER

MRSA in Pig Population

MELDRA IVBULE[1], EDVĪNS MIKLAŠEVIČS[2], LIENE ČUPĀNE[2], LAIMA BĒRZIŅA[3], ANDRIS BĀLIŅŠ[4] and ANDA VALDOVSKA[5]

[1] Food and Veterinary Service, Veterinary Surveillance Department, Riga, Latvia
[2] Riga Stradins University, Institute of Oncology, Riga, Latvia
[3] Latvia University of Agriculture, Faculty of Information Technology, Jelgava, Latvia
[4] Latvia University of Agriculture, Scientific Laboratory of Molecular Biology and Microbiology, Jelgava, Latvia
[5] Latvia University of Agriculture Faculty of Veterinary Medicine, Jelgava, Latvia

Submitted 11 September 2016, revised 13 January 2017, accepted 2 February 2017

Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is widespread worldwide in different types of animal species and as a zoonosis takes a great risk for human health not only as a food toxicoinfection, but also as a highly resistant pathogen causing serious soft tissue infectious, septicaemia and even death. One of the most affected food-producing animal species is swine in the production of which new antibiotics in big amounts are used more and more continuously, increasing antimicrobial resistance. In this study several commercial pig farms and pigs with different age groups as well as farm workers and samples from environment were examined with the purpose of detecting MRSA prevalence and evaluating antimicrobial resistance. A total of 85 isolated MRSA strains were characterised by conventional microbial and molecular methods. MRSA was found in all farms. MRSA prevalence in different pig age groups and farms varied from none to 79.2% reaching higher values among 3–3.5 (26.6%) and 4–4.5 (31.9%) old pigs. The 98.7% of 74 further investigated MRSA isolates were resistant to penicillin, 94.9% to tetracycline, 45.6% to cephalexin and 10 different *spa* types were found among which *spa* type *t011* was the most widespread. To the best of our knowledge, this is the first time MRSA was researched in sow milk and the first description of the presence of MRSA in several age groups of pigs in Latvia.

Key words: *Staphylococcus aureus*, antimicrobial resistance zoonosis, MRSA in pig farms

Introduction

Staphylococcus aureus is an important cause of food poisoning, pneumonia, wound infections and nosocomial bacteraemia for humans (Tiemersma *et al*., 2004). The methicillin resistance of *S. aureus* is mediated by positive *mec*A gene, which encodes penicillin-binding protein 2a (PBP2a) (Chambers, 1997). Among food animals, pigs have been implicated as one source of potential infections to humans, including farmers, slaughterhouse workers, and veterinarians who are in frequent contact with MRSA-colonized pigs (Voss *et al*., 2005; Huijsdens *et al*., 2006; Wulf *et al*., 2008). A subsequent worrisome report indicated that 40% of pigs from the Netherlands carried MRSA CC398 in their nostrils (de Neeling *et al*., 2007; van Duijkeren *et al*., 2008). This observation has been confirmed by a number of studies in other countries, including Belgium (Denis *et al*., 2009), Denmark (Guardbassi *et al*., 2007), Germany (Whitte *et al*., 2007), the USA (Smith *et al*., 2009), and Singapore (Sergio *et al*., 2007). Especially pigs and also pig farmers and their families were found to be colonized with MRSA and in the Netherlands contact with pigs is now recognized as a risk factor for MRSA carriage (Van Duijkeren *et al*., 2008).

In addition, there is rather little knowledge of MRSA carriage related to the age of pigs. Therefore this study is the first description of the presence of MRSA in several age groups of pigs in Latvia. The aim of the study was to find out the occurrence of MRSA in several age groups of pigs, in environment and evaluate antimicrobial resistance and see if there any differences or similarities to other European countries.

Experimental

Materials and Methods

Farm characteristics. During the present study three Latvian pig farms were sampled from October to March. These three farms were selected with different

* Corresponding author: M. Ivbule, Food and Veterinary Service, Veterinary Surveillance Department, Riga, Latvia;
e-mail: Meldra.Ivbule@pvd.gov.lv

Table I
Characteristic of farms.

Cryteria	Farm A	Farm B	Farm C
Number of sows	250	1200	2000
Number of fattening pigs	1500	8000	12000
Batch monitoring systems (weeks)	3	3	3
Weaning age (days)	28	28	30
Separate building with separate air supply	No	Yes	Yes
Sows condition score	2	3.5	3
Suckling piglets condition score	2.5	3.5	3
Fattening pigs condition score	2.5	3.5	3.5
Evidence of scars and purulent lesions	No	No	Yes
Signs of cannibalism	Yes	No	Yes
Reduced fertility (small litter 7–8), weak and lot of stillbirth	Yes	No	No
Dirty, wet cages and pens	Yes	For fattening pigs	Yes, 24°C for suckling piglets
Slatted floors	Yes	Except 4–4.5 month age group	Yes
Lack of straw	Yes	Except 4–4.5 month age group	Yes
Antibiotic usage	For treatment	For treatment and prophylaxis	For treatment

amount of pigs. All three farms were closed pig farms without any other commercially bred farm animals presented and were located in different areas of Latvia. These farms had farrow-to-finish pig production with size varying from 1500 to 12000. Each farmer also completed a questionnaire on farm size, internal and external biosecurity measures and antimicrobial drug use over the preceding 6 months. The characterising of each pig complex is described in Table I. The body condition of swine was scored according to Stockmanship standards (Carr, 1998). Evaluation of animal welfare, hygiene, and microclimate conditions in pig complexes were based on Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs and microclimate standards according to Muirhead (Muirhead *et al*., 2013) suggestions.

Sample collection. Pigs were divided into four groups: pre-weaned piglets with sows, 3–3.5 month old piglets, 4–4.5 month old piglets and fattening pigs (shortly before slaughter) (see Table II). There were collected nasal (n = 305) and rectal (n = 305) samples from all farms. There were taken milk samples (n = 69) and air samples (n = 22). In total amount 305 pigs and 716 microbiological samples were investigated.

Samples were taken from randomly selected healthy pigs. Nasal and rectal samples were collected with sterile transport swabs (Meus, IT). Milk samples were collected in 50 ml amount sterile tubes without preservative. Air samples were collected using Baird-Parker Agar plates according to Koch's sedimentation method (Boucher *et al*., 2010). The number of sampled environment, workers and pigs per age category per farm is shown in Table II. One swab from each worker was taken from both nares. Environmental samples were obtained in every compartment in. All microbiological samples were stored in 4°C and first isolation was made during 24 hours after sample collection.

Microbiological examination. Microbial examination was performed in the Latvia University of Agriculture (LUA), Faculty of Veterinary Medicine. Samples from transport swabs were transferred on Baird-Parker Agar with egg yolk supplement (Becton, Dickinson, USA), and incubated in 37°C for 24 hours according to LVS EN ISO 6888-1:1999 A1:2003 'Microbiology *S. aureus* and other species – Part 1: Technique using Baird-Parker agar medium – Amendment 1: Inclusion of precision data. After incubation positive colonies were inoculated on Mannitol Salt Agar (MSA) plates (Biolife, IT) at 37°C for 24 hours and suspended in Brain Heart infusion (BHI) (Acumedia manufacturers). *Staphylococcus* coagulase tube test (Becton Dickinson,

Table II
Investigated pigs, milk and air samples in each complex.

Group of pigs/sample type	Number of investigated pigs/samples			
	Farm A	Farm B	Farm C	Total
Suckling piglets with sows	32	32	32	96
3-3.5 month old piglets	15	25	24	64
4-4.5 month old piglets	24	24	24	72
Fattening pigs	25	24	24	73
Milk	18	25	26	69
Air	5	9	8	22
Workers	4	4	7	15

USA) was done by using BHI suspension after 24 hours incubation period at 37°C. Coagulase positive samples with positive reaction on MSA plates were determined as *S. aureus*-like and were inoculated on CHROMagar Staph aureus plate (Becton Dickinson, USA) in 37°C for 24 hours. Isolates were confirmed to be *S. aureus* by examining of previous tests. Samples were categorised positive, if at least one *S. aureus* positive colony-forming unit was isolated. Positive colonies from CHROMagar Staph aureus plate were inoculated on CHROMagar MRSA plate (Becton Dickinson, USA). Samples were categorised positive if at least one MRSA positive colony-forming unit was isolated. These samples were categorised as MRSA-like and were stored at –20°C until further use.

MRSA identification. MRSA identification and further examination was performed in Riga Stradins University, Institute of Oncology and in LUA Laboratory of Molecular Biology and Microbiology. One suspected positive MRSA-like colony per sample was then confirmed by PCR and typed by *spa* typing.

Animals and human were considered positive when MRSA was isolated and confirmed with multiplex-PCR form at least one anatomical sampling site. The dominant pig *spa*- and SCC *mec*-type was defined as the type that was most abundantly present in pigs per farm.

DNA was isolated by E.Z.N.A. Bacterial DNA Kit following manufacturer's instructions. DNA amount was verify by ND-1000 spectrophotometer. Polymerase chain reaction (PCR) was performed by HotStarTaq® Plus Master Mix Kit following manufacturer's instructions. The primer sequences for the *mec*A genes were: mecA F: 5'-GTAGAAATGACTGAACGTCCGATGA-3' and *mec*A R: 5'-CCAATTCCACATTGTTTCGGTCTAA-3'. Amplification of DNA was performed in a Applied Biosystems 2720 thermal cycler using the following conditions: initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation (94°C for 1 min), annealing (55°C for 1 min) and extension (72°C for 1 min), following final extension at 72°C for 10 minutes. The amplicons were separated in a 2% agarose gel. After electrophoresis fragments were checked out by UV transilluminator visualization and photographed for visual prove. MecA positive samples were 310 base pair long. *spa* typing was performed as has been described (Shopsin *et al*., 1999). The *spa* gene typing was performed through the Ridom Spa server (www.spaserver.ridom.de).

Antimicrobial susceptibility testing. Randomly selected 74 MRSA positive samples were tested for antimicrobial susceptibility by the disk diffusion method using Oxoid™ (Thermo Scientific) Antimicrobial Susceptibility Disks, following recommendations for Clinical and Laboratory Standards Institute (CLSI) for inoculum preparation, inoculation and incubation (CLSI, 2010). The interpretation of results was done according to the information provided by Thermo Scientific instruction for each type of antibiotic discs. The following antimicrobial agents were tested: Amoxycillin/clavulanic acid (2:1 AMC; 30 μg), Penicillin V (PV; 10 μg), Oxacillin (OX; 1 μg), Cephalexin (CL; 30 μg), Ciprofloxacin (CIP; 5 μg), Tetracycline (10 μg; 30 μg), Clindamycin (DA; 2 μg), Erythromycin (E; 15 μg), Gentamicin (CN; 10 μg), Trimethoprim/sulphamethoxazole 1:19 (Cotrimoxazole) (SXT; 25 μg), Meropenem (MEM; 10 μg), Vancomycin (VA; 30 μg). After 24 h of incubation at 37°C, inhibition zones were measured in millimetres on Mueller-Hinton agar plates (Oxoid, UK) and interpreted according to the manufacturer directions.

Data statistical analysis. Animals and human were considered positive when MRSA was isolated and confirmed with multiplex-PCR form at least one anatomical sampling site. The dominant pig spa- and SCC *mec-type* was defined as the type that was most abundantly present in pigs per farm. Statistical analysis was conducted using software SPSS 16 (SPSS, INC., Chicago, IL, USA). The analysis of contingency tables based on statistics of Chi-square test for independence was performed to determine whether there is a significant association between different farms, slaughterhouses and pig age groups. The Chi-square test was used to analyse whether the different farms or slaughterhouses and pig age groups were related to *S. aureus* and MRSA prevalence. Hypothesis of independence was tested at significance level 0.05. Cramér's V coefficient was used to measure the strength of the association between the variables as post-test after chi-square has determined significance. Cramer's V varies between 0 and 1, showing little association between variables close to 0 and indicating strong association between variables close to 1. Bayes' theorem was used to calculate probability to find staphylococci in samples taken from infected pigs.

Results

We isolated 11.9% MRSA positive samples (85 from 716) samples and identified 10 different *spa* types from all MRSA isolates.

In the present study microorganisms, as shown in Fig. 1, varied significantly (χ^2 p value < 0.05), *S. aureus* prevalence at the farm level ranged from 22.9% to 65.4% and MRSA prevalence ranged from 4.2% to 44.2% The highest prevalence of all staphylococci were seen in Farm C: 65.4% *S. aureus* and MRSA 44.2% positive samples. The lowest prevalence of staphylococci was seen in Farm B: *S. aureus* 22.9% and MRSA 9.5%.

The prevalence of *S. aureus* (Fig. 2.) in different age groups varied from 33.3% in suckling piglets group to 53.4% in 4–4.5 month old piglet group, but prevalence

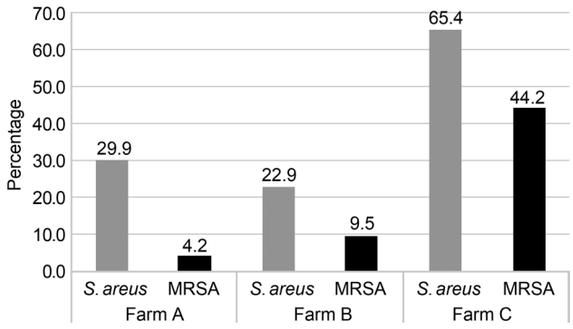

Fig. 1. *S. aureus* and MRSA prevalence in pig farms.

alence of MRSA positive pigs were detected in farm C (44.2%) with the highest evidence in 3–3.5. month age group (70.8%) and in 4–4.5. month age group (79.2%). Only several milk samples were positive- in farm B (4.0%) and in farm C (7.7%). There were no positive MRSA samples taken from environment.

As seen in Fig. 3, 19.7% of all samples were MRSA positive (nasal samples 8.2% and 5.6% rectal samples) and 5.9% of all MRSA positive samples were seen in both rectal and nasal samples. Analysing data we found out, that MRSA and *S. aureus* positive sample distribution depending from sample source is similar. Taking

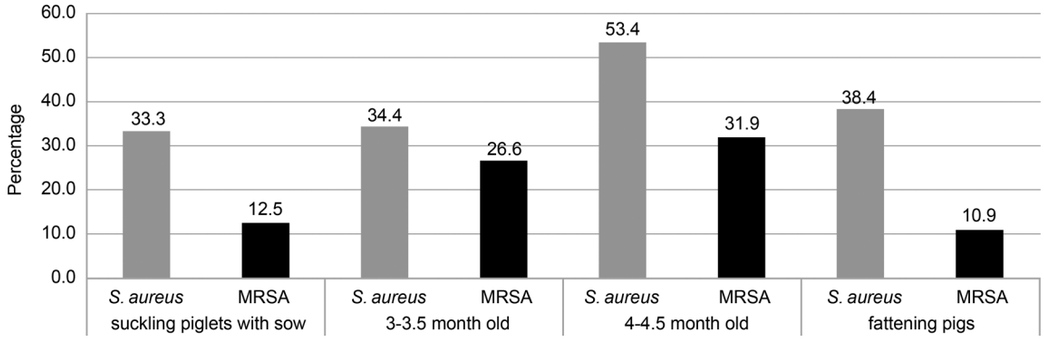

Fig. 2. *S. aureus* and MRSA prevalence between age groups.

of MRSA varied from 10.9% in fattening pig group to 31.9% in 4–4.5 month old pig group. The highest prevalence of MRSA positive samples were seen in 4–4.5 and 3–3.5 month old pigs, but prevalence of *S. aureus* was similar in all age groups except 4–4.5 month age group, where it was for 15.0% to 21.1% higher as in the other groups.

Only 13.3% workers (2 from 15) were MRSA positive. MRSA prevalence in different farms and pig age groups varied from zero to 79.2% (see Table III). In farm A, where the prevalence of MRSA was lower, MRSA positive pigs were found only in 4–45 month (8.3%) and in fattening pig group (8.0%). In farm B MRSA was not detected in 3–3.5 month pig age group, but the highest amounts of positive pigs were seen in suckling piglet group (15.6%). The highest prevonly nasal or rectal samples for MRSA testing decreases probability to find microorganism for 10.2% to 41.7%. According to Bayes' theorem the probability of finding infected pigs with MRSA taking rectal samples is 0.28, nasal samples – 0.42, but in both samples 0.30 and the probability of finding infected pigs with *S. aureus* taking only nasal samples is 0.15, only rectal samples – 0.50, and for both samples – 0.35.

In our study in 7 cases from one animal two different MRSA *spa* types were isolated. There were seen two different MRSA *spa* type combinations: *spa* type *t808* and *t1985* in farm C in 3–3.5 month age group and 4–4.5 month age group.

We isolated 74 MRSA isolates with 10 different *spa* types (see Table IV and Table V). MRSA distribution depending from *spa* type, sample origin and resistance

Table III
MRSA prevalence in farms.

Farm	Suckling piglets with sow*		3–3.5 month old*		4–4.5 month old*		Fattening pigs*		Total prevalence	Milk*		Workers*	Environment*
A	0/32	0	0/15	0	2/24	8.3%	2/25	8.0%	4.2%	0/18	0	2/4	0/5
B	5/32	15.6%	0/25	0	2/24	8.3%	3/24	12.5%	9.5%	1/25	4.0%	1/4	0/9
C	7/32	21.9%	17/24	70.8%	19/24	79.2%	3/24	12.4%	44.2.%	2/26	7.7%	0/7	0/8
Total	12/96	12.5%	17/64	26.6%	23/72	31.9%	8/73	10.9%	18.7%	3/69	4.3%	3/15	0/22

* MRSA positive samples from all tested

Table IV
MRSA origin.

Spa type	Farm	Number of Antimicrobial resistance profile	Antimicrobial resistance profile	Origin (n)
t011	C	2	Pen-AmCl-Tetr	Fattening pigs (n = 1), 3–3.5 month old (n = 2)
		3	Pen-AmCl-Cip-Tetr	Suckling piglets (n = 1)
		4	Pen-AmCl-Cef-Tetr	4–4.5 month old (n = 3)
		8	Pen-AmCl-Cef-Tetr-Clin-Ery-Ge-Tri-Me-	4–4.5 month old (n = 1)
		11	Pen-Cef-Tetr	4–4.5 month old (n = 13), suckling piglets (n = 1), sows (n = 2), milk (n = 1)
		12	Pen-Cef-Tetr-Clin	4–4.5 month old (n = 1)
		13	Pen-Cef-Tetr-Me	4–4.5 month old (n = 1)
		20	Pen-Tetr	Fattening pigs (n = 1), 4–4.5 month old (n = 5), 3–3.5 month old (n = 11), suckling piglets (n = 2)
		21	Tetr	4–4.5 month old (n = 1)
	A	4	Pen-AmCl-Cef-Tetr	4–4.5 month old (n = 1)
	B	4	Pen-AmCl-Cef-Tetr	Suckling piglets (n = 1)
		11	Pen-Cef-Tetr	Suckling piglets (n = 1)
		23	Pen-Cef-Tetr-Clin-Ery	Fattening pigs (n = 1)
t1333	B	1	Pen	Fattening pigs (n = 1)
		24	Pen-Tetr-Ery	Fattening pigs (n = 1)
		25	Pen-Tetr-Clin-Ery	4–4.5 month old (n = 1)
t808	C	4	Pen-AmCl-Cef-Tetr	3–3.5 month old (n = 1)
		20	Pen-Tetr	4–4.5 month old (n = 3), 3–3.5 month old (n = 1)
t899	A	1	Pen	Fattening pigs (n = 1)
		20	Pen-Tetr	4–4.5 month old (n = 1)
		5	Pen-AmCl-Tetr-Tri-Me	Fattening pigs (n = 1)
t400	B	1	Pen	milk (n = 1)
		8	Pen-AmCl-Cef-Tetr-Clin-Ery-Ge-Tri	sow (n = 1)
		20	Pen-Tetr	sow (n = 1)
		25	Pen-Tetr-Clin-Ery	Suckling piglets (n = 1)
		26	Pen-Tetr-Clin	4–4.5 month old (n = 1)
		27	Pen-Cef-Tetr-Clin-Ery-Ge	Suckling piglets (n = 1)
t1580	C	11	Pen-Cef-Tetr	Fattening pigs (n = 1)
t1985	C	11	Pen-Cef-Tetr	Suckling piglets (n = 1)
		13	Pen-Cef-Tetr-Me	3–3.5 month old (n = 1)
		20	Pen-Tetr	Suckling piglets (n = 1), 3–3.5 month old (n = 1)
t693	A	13	Pen-Cef-Tetr-Me	Worker at farm (n = 1)
t2383	C	16	Pen-Tetr-Clin-Ge	4–4.5 month old (n = 1)
t1255	B	11	Pen-Cef-Tetr	Farm worker (n = 1)

* Pen-penicillin, AmCl – amoxicillin with clavulanic acid, Cef – cephalexin, Cip – ciprofloxacin, Clin – clindamycin, Ery – erythromycin, Ge – gentamycin, Me – meropenem, Tetr – tetracycline, Tri – trimethoprim sulphonamide.

against several antibiotics are shown in Table IV. The amounts of different *spa* types were higher in farms with greater pig production (Farm C) and higher MRSA prevalence (Farm C). We found 3 *spa* types in farm A, in farm B – 4, but in farm C – 5 *spa* types. MRSA *spa* type *t011*, that was one of the most widespread (51 from 74), which was evident in all three farms while other *spa* types were seen in only one farm (Table V). Analysing MRSA strains, we found out, that spa types *t899* belong to *ST9*, *t400* to *ST528*, but *t011* to *ST398*.

Antimicrobial susceptibility testing on the selected MRSA isolates revealed the presence of 19 different antibiotic profiles. Amounts of MRSA *spa* type distribution among samples are shown in Table VI and Fig. 4.

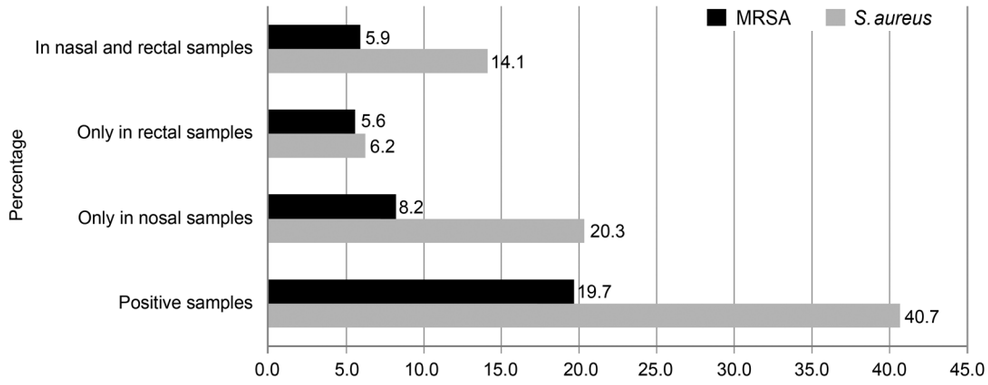

Fig. 3. *S. aureus* and MRSA findings depending from sample type.

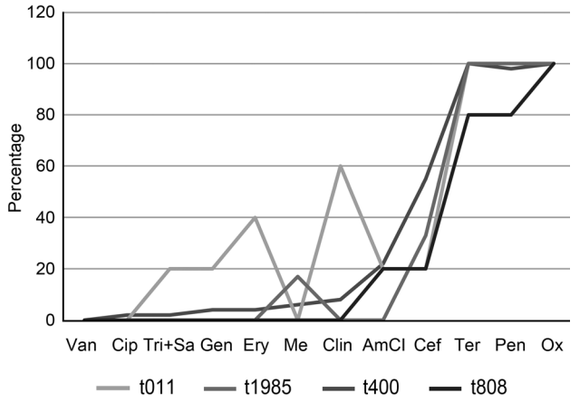

Fig. 4. Antimicrobial resistance of four MRSA most spread *spa* types.

As seen from our study 69% of MRSA isolates belong to *spa* type *t011* (n = 51), and other most widespread *spa* types are *t808* (7%, n = 5), *t1985* (5%, n = 4) and *t400* (5%, n = 4). Two to eleven different antibiotic resistance profiles were seen depending on MRSA *spa* type (Table VI). The most spread *spa* types also were the ones with the highest antibiotic profile heterogeneity, for example *spa* type *t011* integrated in to more than one half (n = 10) of antibiotic profiles (Table VI), while the lowest heterogeneity was evident among MRSA *spa* type *t808* (n = 2). MRSA isolate t1255 from farm worker, which belongs to MRSA isolates from pig origin, had the highly widespread antibiotic type Pen-Cef-Tetr.

Sixty-four percent of the isolates belonged to the two most prevalent antibiotic resistance profiles (Table VII).

MRSA *spa* type *t011* was almost evident in all most frequently spread antibiotic resistance profiles, but *spa* type *t1985* was evident in antibiotic type Pen-Tetr, Pen-Cef-Tetr and Pen-Cef-Tetr-Me. Most of all MRSA isolates showed multidrug resistance.

The highest antimicrobial resistance was seen against four (34% of all MRSA isolates), three antibiotics (32.9% of all MRSA isolates) and five (17.7% of all MRSA isolates) antibiotics. Lower amounts of isolates were resistant to six (5.1%) and seven (1.3%) antibiotics.

98.7% of all MRSA isolates were resistant to penicillin, 94.9% to tetracycline, 45.6% to cephalexin, 19.90%

Table V
MRSA spa type distribution in farms.

Farm	*Spa* types
A	*t011* (n = 1), *t899* (n = 3), *t693* (n = 1)
B	*t011* (n = 3), *t1333* (n = 3), *t400* (n = 4), *t1255* (n = 1)
C	*t011* (n = 47), *t808* (n = 5), *t1580* (n = 1), *t1985* (n = 4), *t2383* (n = 1)

Table VI
Characterisation of most frequently spread MRSA spa types.

Spa type	% of all MRSA isolates	The highest resistance for current antibiotics	Total amount of different antimicrobial resistance profiles
t1985	5	Pen, Tetr, Cef, Me	3
t011	69	Pen, Tetr, Cef, Am-Clav	10
t808	7	Pen, Tetr, AM Cl, Cef	2
t400	5	Pen, Tetr, Clin, Ery	4

Table VII
Characterisation of most frequently found antibiotic types.

Antibiotic types most frequently found	Number of MRSA isolates	% from all MRSA isolates	Number of different MRSA *spa* types
Pen-Tetr	28	38	5 (*t011, t808, t899, t400, t1985*)
Pen-Cef-Tetr	19	26	4 (*t011, t1580, t1985, t1255*)
Pen-AmCl-Cef-Tetr	6	8	2 (*t011, t808*)
Pen	3	4	3 (*t1333, t899, t400*)
Pen-AmCl-Tetr	3	4	1(*t011*)
Pen-Cef-Tetr-Me	3	4	3 (*t011, t1985, t1255*)

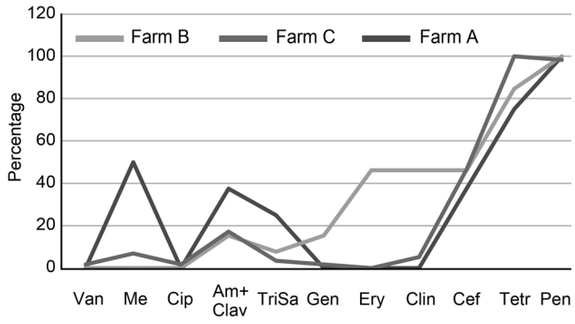

Fig. 5. Antimicrobial resistance in farms.

to amoxicillin combined with clavulanic acid, 11.4% to clindamycin, 10.0% to meropenem, 7.6% to erythromycin, 6.3% to trimethoprim sulphonamide, 3.8% to gentamycin and 1.3% to ciprofloxacin.

All isolates were sensitive to vancomycin, but 9 MRSA isolates were intermediate to vancomycin, *t011* (n = 5), *t693* (n = 1), *t899* (n = 2) and *t400* (n = 1).

Comparing MRSA *spa* type antibiotic resistance, we found out, that even between one *spa* type, there are differences in antibiotic resistance profiles, therefore we calculated average % resistance from all isolated one type MRSA *spa* types and showed results in graphics (Fig. 4).

All four most prevalent *spa* types in our samples differ each from another, but a common tendency can be seen, that is that all these types are almost 80% to 100% resistant to tetracycline and penicillin. *spa* type *t011* is the most widespread compared to other *spa* types, but has a moderate antibiotic resistance profile, while *t400* is mostly resistant to seven of the 12 tested antibiotics.

Among all the most widespread MRSA *spa* types can be found isolates that are more or less, resistant to penicillin, cephalexin and tetracycline. MRSA resistance was seen even to antibiotics that are not frequently used or are not allowed to be used for food chain animal treatment, such as, gentamycin, ciprofloxacin, cephalexin and meropenem. In some rare cases MRSA isolates showed (not showed in figures) intermediate sensitivity to vancomycin.

Comparing antibiotic resistance in farms (Fig. 5), greater resistance appears against meropenem and amoxicillin combined with clavulanic acid in Farm A, but in Farm B there is greater resistance against gentamycin, erythromycin and clindamycin. However, there is no evident difference in antibiotic resistance among farms related to farm size and pig number.

Discussion

This study investigated the transmission and distribution of *S. aureus* and MRSA in individual pigs throughout the production cycle, environment and pig industry workers. Significant findings include the identification and detailed characterisation for the first time of MRSA isolates from Latvian pigs and demonstrated MRSA colonization status between several pig age groups. In addition, this study studied MRSA distribution and antibiotic resistance level tendencies between different pig farms depending from production amounts. It documented tendency in the MRSA distribution and antibiotic resistance profiles according to MRSA *spa* types. Antimicrobial use is also thought to be a factor in the emergence and transmission of MRSA in pigs and deserves further investigation.

However, it is acknowledged that many factors, in addition to pig colonisation status in farms, are likely to be important influences on carrier status. Such factors might include antimicrobial medication for treatment and prophylaxis, animal welfare aspects and pig density and contact availability to each another. In our study MRSA was not found in environmental samples, but two *S. aureus* isolates were found (two from air in 3–3.5 old pig group in farm C, where the MRSA prevalence in pigs was higher among all farms), but other investigations have shown MRSA distribution in barn spaces in Germany (Friese *et al.*, 2012) MRSA appears in 23 of 27 investigated pig barns (85.2%) and the prevalence in dust samples appeared 100% whereas in EFSA report (EFSA, 2009c) the prevalence in dust samples was 0%. In other studies testing dust and farm air were used filtration methods using specific equipment, but we used Koch's sedimentation method, and it could be a reason for such a low MRSA detection level. Failure to detect airborne MRSA and *S. aureus* in farms by the used Koch's sedimentation method in our study does not guarantee the absence of these bacteria in the air.

MRSA appearing in air samples reveals the difficulties in reducing the spread of bacteria within an animal house. It can be concluded that very effective cleaning and disinfection of the stables including all ventilation systems before stocking with new pigs is necessary to avoid transmission of MRSA between subsequent fattening groups of animals within breeding farms by contaminated premises. Depending on the ventilation system and construction of the industrial house the dissemination of MRSA through the whole building *via* air seems possible. Occurrence of MRSA in the air may lead also to colonisation of negative animals without direct contact with MRSA carriers (Friese *et al.*, 2012).

In our study the prevalence of *S. aureus* and MRSA varied in each age group. The results indicated differences between the farm types with respect to within farm associated MRSA *spa* type. The average MRSA prevalence in farms (4–44.2%) were little bit lower or similar to other studies in Italy (EFSA, 2009; Batisti *et al.*, 2010) where MRSA prevalence in pig herds

warried from 38% to 52%, in Belgium (Pletnickx *et al*., 2013) 40–84%. Moreover, the holding size was found to be a significant factor influencing the prevalence (Battisti *et al*., 2010). Larger farms have showed a higher risk.

This study found the average carriage rate of MRSA was at its highest in 3–3.5 and 4–4.5 old pigs. Burns with co-authors (2014) found the average carriage rate of *S. aureus* was at its highest on day 2 after farrowing, followed by a decrease prior to weaning and similar findings were reported by other authors (Smith *et al*., 2009; Weese *et al*., 2010; Broens *et al*., 2011; Verhegghe *et al*., 2011). In our study the prevalence of MRSA and *S. aureus* was highest in 3–3.5 and 4–4.5 month old pigs and decreased among fattening pigs, but in Burns study with co-authors (2014) the prevalence of MRSA continued to increase during the 100 day investigation period. According to Weese (2010) and Dewale (2013) increase of MRSA positive pigs recorded at weaning was due to the commingling of positive and negative pigs, stress during weaning, age related susceptibility and contamination of other sites on farms. In our study depending from farms pigs in 3–4.5 month age were moved to fattening buildings and once again regrouping was carried out and different holding conditions appeared causing additional stress. Weaning, regrouping and moving may be a point at which controls could be implemented in order to reduce the transmission of MRSA.

Burns and co-authors have stated (2014) that more than 1/3 of *S. aureus* isolates were resistant to tetracycline and erythromycin, a similar situation to that seen in our study, moreover 46% of MRSA isolates were resistant to cephalexin and 11% to clindamycin that were not used for pig treatment in farms, but resistance to penicillin, that were used us a first choice antibiotic several years ago and tetracycline that were administrated in these farms during the sample taking process, reached 99% and 95%. Antibiotic usage for prophylactic purposes does not decrease MRSA distribution. The MRSA distribution in farm B, where antibiotics are used for prophylactic purposes, are little lower as in Farm C, where antibiotics were used for treatment only but quite higher than in farm A, where also antibiotics were used for treatment purposes only. Similar parallels from our study to Italian study (Normanno *et al*., 2015) are seen in antibiotic resistance profiles, where mostly MRSA isolates were multidrug resistant, including resistance to clindamycin, tetracycline, erythromycin. A study from Denmark (Witte *et al*., 2007) showed that *spa* type *t034* were the most widespread, whereas other authors (Tenhagen *et al*., 2009; Broens *et al*., 2011; Crombe *et al*., 2012; Friese *et al*., 2012; Pletinckx *et al*., 2013) and our study most frequently found *spa* type *t011*, that was isolated from all farms. MRSA *t011 spa* type was also found in Belgium poultry farm (Nemati *et al*., 2008), in Denmark (Agerso *et al*., 2012), in Italy (Normanno *et al*., 2015). *spa* type "t899" that we detected in our study, was found in Normanno's research (Normanno *et al*., 2015). According to Fishers' test value (1.1×10^{-11}) statistically significant differences were found regarding the origin of samples, and MRSA *spa* types isolated from farms. One another *spa* type *t1333* that was found in our study, was one of the most frequently found MRSA *spa* type in a study by Tehnagen and co-authors (2009). Our study agrees with Pletinckx and co-authors (2013), where different antimicrobial resistance profiles per *spa* type and per farm appear.

Human infectious caused by MRSA are one of main causes of morbidity and mortality in industrialized countries. In addition to the traditional routes of MRSA infection, it has recently been demonstrated that direct transmission to humans takes a place *via* contact with farm animals (Wendlandt *et al*., 2013). A lot of studies on spread of MRSA in farm animals and their carcases have included pigs, which are currently the most important reservoir of MRSA (Gomez-Sanz *et al*., 2010; Overesch *et al*., 2011).

The high presence of MRSA in pigs is a potential professional hazard for these working in the meat production chain (workers in farms and slaughterhouses, transportation workers and veterinarians). It is known that people working several hours per week in direct contact with pigs colonized or infected with MRSA animals are exposed to high risk of nasal colonization (Voss *et al*., 2005; van Loo *et al*., 2007; Witte *et al*., 2007; Denis *et al*., 2009; Moodley *et al*., 2011). The general population shows a high prevalence (approximately 30%) of *S. aureus* nasal colonization, whereas MRSA nasal colonization levels are lower (0.7–1.5%) depending on geographical area (Wertheim *et al*., 2004; Gorwitz *et al*., 2008; Munckhof *et al*., 2009). Human colonization implies that carriers become a staphylococcal reservoir and may transfer the infection to others or animals, especially pigs. In addition, subclinical carriage of MRSA by humans is considered a risk factor for subsequent occurrence of clinical disease (Cohn and Middleton, 2010; Jordan *et al.* 2011). Many studies have investigated MRSA nasal colonization among personnel in contact with animals, especially farmers and veterinarians (van Cleef *et al*., 2014). In our survey, the anterior nares 1 out of 15 investigated workers from farms were colonised by MRSA, that were higher than in other studies: in China no MRSA in 107 slaughterhouse workers (Cui *et al*., 2009) in Switzerland no MRSA in 179 slaughterhouse workers (Huber *et al*., 2010), in Netherlands 5.6% of nasal carriage from 195 pigs in slaughterhouse, which was higher than the prevalence 0.1 among the general population of the country (van Cleef *et al*., 2010). High con-

centration of MRSA in barns may also be an issue of occupation health. It has been proved in several studies that humans working in pig farms carry MRSA of the same sequence type as swine (Cunny *et al*., 2009; Van Den Broek *et al*., 2009). Considering the MRSA and *Staphylococcus aureus* occurrence in worker nares sample, this fact is not surprising since the stuff do not wear any respiratory masks and are in close physical contact with pigs. In addition, their hands, equipment, clothes and boots can serve as infection transmitters and contaminants.

Antimicrobial resistance is increasing worldwide in human bacterial pathogens and zoonotic agents and this may cause a risk for effective treatment of infections in humans. Multidrug resistance was prevalent in our MRSA isolates in all groups – from pigs and worker in farms. Most of all isolates displayed resistance to two or more classes of antimicrobials and some of them were resistant or with intermediate sensitivity to vancomycin, that indicate development of resistance to that antibiotic. These findings are in agreement with other studies of high MRSA prevalence and antimicrobial resistance in isolates from pigs, pork and humans (Batisti *et al*., 2010; Jackson *et al*., 2013). As expected 99% of isolates were resistant to penicillin and 95% to tetracycline, but quite high resistance appears to cephalexin (46%), amoxicillin combined with clavulanic acid (10%), clindamycin (11.4%), meropenem (10%) and erythromycin (7.6%). Several studies have showed 100% resistance to MRSA isolates (van Duijkeren *et al*., 2008; Batisti *et al*., 2010; Fesler *et al*., 2012; Crombe *et al*., 2013) and Pletinckx (2013) have found high resistance to trimethoprim, lincomycin and ciprofloxacin.

Finally, it is known that MRSA prevalence and *spa* types differ according to farm density and animal welfare conditions. In farms with higher amount of pigs, several evident scars and purulent lesions on joints MRSA prevalence and thought different MRSA spa types were found higher as in others. The pigs used in our study originated from different farms in our country, and this could be the reason for the wide heterogeneity of the MRSA *spa* types we found. An unexpected finding was that in farms, where pig condition score was lower and reduced sow fertility was in presence, staphylococcal colonisation rate was lower than in farms where pig condition score were higher and better animal welfare conditions were evident. In addition no significantly lower staphylococcal colonisation in farm B, compared to other farms, were seen, despite antibiotic usage for prophylactically purposes.

As far as we know, this is the first report documenting the prevalence and characteristics of MRSA in farms and stuff involved in pig industry in Latvia and MRSA detection in sow milk.

Literature

Agerso Y., H. Hasman, L.M. Cavaco, K. Pedersen and F.M. Aarestrup. 2011. Study of methicillin resistant *Staphylococcus aureus* (MRSA) inn Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. *Vet. Microbiol.* 157: 246–250.

Batisti A., A. Franco, G. Merialdi, H. Hasman, M. Juresica, R. Lorenzetti, F. Feltrin, M. Ini and F.M. Aerestrup. 2010. Heterogenity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. *Vet. Microbiol.* 142: 361–366.

Broens E.M., E.A.M. Graat, P.J. Van DerWolf, A.W. Van De Giessen and M.C.M. De Jong. 2011. Transmission of methicillin resistant *Staphylococcus aureus* among pigs during transportation from farm to abbatoir. *Vet. J.* 189: 302–305.

Carr J. 1998. *Garth Pig Stockmanship Standards*. 5M Enterprises, London.

Chambers H.F. 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin. Microbiol.* 10: 781–791.

Cohn L.A. and J.R.A. Middleton. 2010. Veterinary perspective of methicillin-resistant staphylococci *J. Vet. Emerg. Crit. Care.* 29: 31–45.

Crombe F., G. Willems, M. Dispas, M. Hallin, O. Denis, C. Suetens, B. Gordts, M. Struelens and P. Butaye. 2012. Prevalencce and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* among pigs in Belgium. *Microb. Drug. Resist*. 18: 25–131.

Cunny C., C. Stanek and W. Witte. 2009. MRSA from the point of view of the RKI: evidence in humans and other animals – a coming zoonosis? (in German). *Dtsch. Tierarzaltz. Wochensschr.* 116: 284–290.

Cui S., J. Li1, C. Hu1, S. Jin1, F. Li, Y. Guo, L. Ran and Y. Mal. 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aure*us from swine and workers in China. *J. Antimicrob. Chemother.* 64: 680–683.

Denis O., C. Suetens, M. Hallin, B. Catry, I. Ramboer, M. Dispas, G. Willems, B. Gordts, P. Butaye and M.J. Struelens. 2009. Methicillin-resistant *Staphylococcus aureus ST398* in swine farm personnel, Belgium. *Emerg. Infect. Dis.* 15(7): 1098–1101.

De Neeling A.J., M.J. Van Broek, E.C. Spalburg, M.G. Van Santen-Verheuvel, W.D. Dam-Daisz, H.C. Boshuizen, A.W. Ven De Giessen, F. Van Duijkeren and H.W. Huijsdens. 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet. Microbiol.* 122: 366–372.

Dewaele I., I. Man, P. Delputte, P. Butaye, G. Vlaemynck, L. Herman, M. Heyndrich and G. Rasschaert. 2013. Methicillin-resistant *Staphylococcus aureus* (MRSA) on Belgian pig farms. http://www2.llu.lv/research_conf/Formatting.pdf/, 2014.03.02.

EFSA. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU. 2008. *EFSA J.* 7: 1376.

Fesler A.T., R.G.M. Riekerink, A. Rothkamp, K. Kadlec, O.C. Sampimon, T.J.G.M. Lam and S. Schwarz. 2012. Characterization of methicillin-resistant *Staphylococcus aureus CC398* obtained from humans and animals on dairy farms. *Vet. Microbiol.* 160: 77–84.

Friese A., J. Schulz, L. Hoehle, A. Fetsch, B.A. Tehnagen, J. Hartung and U. Roesler. 2012. Occurrence of MRSA in air and housing environment of pig barns. *Vet. Microbiol.* 158: 129–135.

Gomez-Sanz E., C. Torres, C. Lozano, R. Fernandez-Perez, C. Aspiroz, F. Ruiz-Larrea and M. Zarazaga. 2010. Detection, molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus CC398* and *CC97* in Spanish slaughter pigs of different age groups. *Foodborne Path. Dis.* 7: 1269–1277.

R.J. Gorwitz, D. Kruszon-Mora, S.K. McAllister, G. McQuillan, L.K. McDougal, G.E. Fosheim, B.J. Jensen, G. Kilgore, F.C. Tenover and M.J. Kuehnert. 2008. Changes in the prevalence of nasal colonization of *Staphylococcus aureus* in United States, 2001–2004. *J. Infect. Dis.* 197: 1226–1234.

Guardabassi L., M. Stegger and R. Skov. 2007. Retrospective detection of methicillin resistant and susceptible *Staphylococcus aureus ST398* in Danish slaughter pigs. *Vet. Microbiol.* 122: 384–386.

Huber H., S. Koller, N. Giezendanner, R. Stephan and C. Zweifel. 2010. Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. *Euro Surveil.* 15: 9642.

Huijsdens X.W., van B.J. Dijke, E. Spalburg, M.G. van Santen-Verheuvel, M. Heck, G.N. Pluister, A. Voss, W.J.B. Wannet and A.J. Neeling. Community-acquired MRSA and pig-farming. 2006. *Ann. .Clin. Microbiol. Antimicrob.* 10: 275–281.

Jackson C.R., J.A. Davis and J.B. Barrett. 2013. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. *J. Clin. Microbiol.* 51: 1199–1207.

Jordan D., J. Simon, S. Fury, S. Moss, P. Giffard, M. Maiwald, P. Southwell, M.D. Barton, J.E. Axon, S.G. Morris and others. 2011. Carriage of methicillin-resistant *Staphylococcus aureus* by veterinarians in Australia. *Aust. Vet. J.* 89: 152–159.

Moodley A, F. Lantronico and L. Guardbassi. 2011. Experimental colonization of pigs with methicillin-resistant *Staphylococcus aureus* (MRSA): insights into the colonization and transmission of livestock-assiciated MRSA. *Epidemiol. Infect.* 139: 1594–1600.

Muirhead M.R. and J.L.T. Aleksander. 2013. *Managing pig health: a reference for the farm.* 2nd edition, Dr. J. Carr, London.

Munckhow W.J., G.R. Nimmo, J.M. Schooneveldt, S. Schlebusch, A.J. Stephens, G. Williams, F. Huygens and O. Giffard. 2009. Nasal carriage of *Staphylococcus aureus*, including community-associated methicillin-resistant strains, in Queensland adults. *Clin. Micribiol. Infect.* 15: 149–155.

Nemati M., K. Hermans, K. Lipinska, U. Denis, O. Deplano, M. Struelens, L.A. Devriesa, F. Pasmans and F. Haesebrouck. 2008. Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry: first detection of livestock-associated methicillin-resistant strain *ST398*. *Antimicrob. Agents Chemother.* 52: 3817–3819.

Normanno G., A. Dambrosio, V. Lorusso, G. Samolis, P. Taranto and A. Parisi. 2015. Methicillin-resistant *Staphylococcus aureus* (MRSA) in slaughtered pigs and abattoir in Italy. *Food Microbiol.* 51: 51–56.

Overesch G., S. Butner, A. Rossano and V. Perreten. 2011. The increase of meyhicillin-resistant *Staphylococcus aureus* (MRSA) and the presence of an unusual sequence type *ST49* in slaughtered pigs in Switzerland. *BMC Vet. Res*. 7: 30.

Pletinckx L., M. Verhegghe, F. Crombe, J. Dewulf, Y. De Blecker, G. Rasschaert, P. Butaye, B.M. Goddeeris and I. De Man. 2013. Evidence of possible methicillin-resistant *Staphylococcus aureus ST398* spread between pigs and other animals and people residing on the same farm. *Preven. Vet. Med.*109: 293–303.

Smith T.C., M.J. Male, A.L. Harper, J.S. Kroeger, G.P. Tinkler, E.D. Moritz, A.W. Capuano, L.A. Herwaldt and D.J. Diekema. 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain *ST398* is present in Mildwestern USA. Swine and swine workers. *PLoS ONE* 4: 4258.

Sergio D.M., T.H. Koh, L.Y. Hsu, B.E. Ogden, A.L. Goh and P.K. Chow. 2007. Investigation of meticillin-resistant *Staphylococcus aureus* in pigs used for research. *J. Med. Microbiol*. 56: 1107–1109.

Tenhagen B.A., A. Fetsch, A.B. Stuhrenberg, G. Schleuter, B. Guerra, J.A. Hammerl, S. Hertwig, J. Kowall, U. Kampe, A. Schroeter and others. 2009. Prevalence of MRSA types in slaughter pigs in different German abattoirs. *Vet. Rec.* 165: 589–593.

Tiemersma E.W., S.L. Bronzwaer, O. Lyytikäinen, J.E. Degener, P. Schrijnemakers, N. Bruinsma, J. Monen, W. Witte and H. Grundman. 2004. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. *Emerg. Infect. Dis.* 10: 34.

van Loo I.H.M., B.M.W. Diederen, P.H.M. Savelkoul, J.H.C. Wounderberg, R. Rosendaal, van A. Belkum, N. Lemmans-den Toom, C. Venhulst, P.H.J. van Keulen and J.A.W. Kluytmans. 2007. Methicillin-resistant *Staphylococcus aureus* in meat products. *Emerg. Infect. Dis.* 13: 1753–1755.

van Cleef B.A.G.L., B.H.B. van Benthem, E.J.M. Verkade, M. van Rijen, M.F.Q. Kluytsmans- van den Bergh, L.M. Schouls, B. Duim, J.A. Wagenaar, H. Graveeland, M.E.H. Bos and others. 2014. Dynamics of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* carriage in pig farmers: a prospective cohort study. *Clin. Microbiol. Infect.* 20: 711–764.

van Cleef B.A.G.L., E.M. Broens, A. Voss, X.W. Huijsdens, L. Zuchner, B.H.B. Van Bethem, J.A.J.W. Kluytsmans, M.N. Mulders and A.W. Van De Giessen. 2010. High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in the Netherlands. *Epidemiol. Infect.* 138: 756–763.

Van Den Vroek I.V., B.A. Cleef, A. Haenen, E.M. Broens, P.J. Van Der Wolf, M.J. Van Deen Broek, X.W. Huijsdens, J.A. Kluytsmans, A.W. Van De Giessen and others. 2009. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. *Epidemiol. Infect.* 137: 700–708.

Van Duijkeren E., R. Ikawaty, M.J. Broekhuizen-Stins, M.D. Jansen, E.C. Spalburg, A.J. De Neeling, J.G. Allaart, A. Van Nes, J.A. Wagenaar and A.C. Fluit. 2008. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. *Vet. Microbiol.* 126: 383–389.

Verhegghe M., L.J. Pletinckx, F. Crombe, S. Vam Weyenberg, F. Haesebrouck, P. Butaye, M. Heyndrickx and G. Rasschaert. 2011. Cochort study for presence of livestock-associated MRSA in piglets: effect of sow status at farrowing and determination of the piglet colonization age. *Vet. Microbiol.* 162: 679–686.

Voss A., F. Loeffen, J. Bakker, C. Klaasen and M. Wulf. 2005. Methicillin resistant *Staphylococcus aureus* in pig farming. *Emerg. Infect. Dis.* 11: 1965–1966.

Weese J.S., A. Zwambag, T. Rosendal, R. Reid-Smith and R. Friendship. 2010. Antimicrobial resistance in staphylococci from animals with particular reference to bovine *Staphylococcus aureus*, porcine *Staphylococcus hyicus,* and canine *Staphylococcus intermedius*. *Vet. Res.* 32: 341–362.

Wendlandt S., S. Schwarz and P. Silley. 2003. Methicillin resistant *Staphylococcus aureus*: a food-borne pathogens? *Ann. Rev. Food Sci. Technol.* 4: 117–139.

Wertheim H.F., M.C. Vos, H.A. Boelens, A. Voss, C.M. Vandenbroucke-Grauls, H.M. Meester, J.A. Kluytmans, P.H. ven Keulen and H.A. Verbrugh. 2004. Low prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) at hospital admissions in the Netherlands: the value of search and destroy and restrictive antibiotic use. *J. Hosp. Infect*. 56: 321–325.

Witte W., B. Strommenger, C. Stanek and C. Cunny. 2007. Methicillin-resistant *Staphylococcus aureus ST398* in Humans and Animals. *Cent. Eur* 13: 255–258.

Wulf M. and A. Voss. 2008. MRSA in livestock animals – an epidemic waiting to happen? *Clin. Microbiol. Infect.* 14: 519–521.