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Presence of methicillin-resistant *Staphylococcus aureus* in slaughterhouse environment, pigs, carcasses, and workers

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a highly resistant and difficult to cure zoonotic microorganism, which makes up a large part of food toxic infections and has shown high prevalence among pig population all over the world. The aim of the study was to establish the occurrence of MRSA in slaughterhouses, evaluate its antimicrobial resistance, and verify whether there are any differences or similarities with reference to other European countries. **Material and Methods:** A total of 100 pigs, 105 carcasses, 19 workers, and 24 samples from the environment of several slaughterhouses were examined by conventional microbial and molecular methods. **Results:** In total, 78 MRSA isolates were found. MRSA prevalence in slaughtered pigs varied from 8.0% to 88.6% depending on the slaughterhouse, reaching higher prevalence in slaughterhouses with higher slaughter capacity. In total, 21.1% of all workers were carriers of MRSA and 6.7% of carcasses were contaminated with MRSA. The 98.2% of MRSA isolates were resistant to penicillin, 89.1% to tetracycline, 60.1% to erythromycin, 65.5% to gentamycin, and 15 different *spa* types were found, among which *spa* type *t01333* was most widespread. **Conclusion:** The study indicated that MRSA prevalence and *spa* types differed according to slaughterhouse slaughter capacity and good hygiene practices. Quite high MRSA occurrence among slaughterhouse workers is one of the main factors which increase pork contamination risk.

Keywords: pigs, slaughterhouses, *Staphylococcus aureus*, antimicrobial resistance.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been increasingly prevalent in pigs, veal, and pig farmers over the last decade (28). MRSA colonisation has recently been identified in people working with pigs, raising concerns about the role of pigs as reservoirs of MRSA for human infection.

MRSA is a multidrug-resistant Gram-positive bacterium, a critically important pathogen in human medicine. MRSA strains are resistant to all β-lactam antimicrobials through a penicillin binding protein (PBP2a) which has a low affinity to all β-lactams. The protein is encoded by the *mecA* gene, which resides on

a mobile genetic element called a staphylococcal chromosomal cassette (SCCmec) lactams. Worldwide, the strains are resistant to a wide range of antibiotics. A subsequent worrying report indicated that 40% of pigs from Holland carried MRSA CC398 in their nostrils (8). This observation has been confirmed by a number of studies in other countries including Belgium (9), Denmark (13), Germany (32), USA (26), and Singapore (2). In Dutch abattoirs, 39% (209/540) of pigs and 81% (44/54) of slaughter batches were MRSA positive in 2005–2006. The higher prevalence of MRSA in pigs in abattoirs compared with the prevalence on farms is evident (8). The occurrence of MRSA in food products and the role of food products

in transmission of MRSA to humans are unknown. One of the debatable issues is whether carcasses obtained from MRSA-colonised pigs could cross-contaminate pork meat and be a source of human infections. Therefore, this study was undertaken to first describe the presence of MRSA in slaughterhouses, and to demonstrate the difference between slaughter techniques and slaughter capacity, as well as to verify which source is the most important for carcass contamination – human, animal, or environmental. The aim of the study was to determine the occurrence of MRSA in slaughterhouse environment, evaluate its antimicrobial resistance, and to establish whether there are any differences or similarities to other European countries.

Material and Methods

Slaughterhouses. During this study, samples were collected from four Latvian pig slaughterhouses (A, B, C, D) from October to March 2014. These slaughterhouses were of different pig slaughter capacity and were located in different areas of Latvia.

Slaughterhouse workers. The number of eligible workers per slaughterhouse was 5 to 10. Slaughterhouses work only three to five days a week. The eligible population preferably included all workers, starting from the main slaughterline (lairage area, scalding/singeing and dehairing area, and evisceration area). Participants who agreed to take part in the study received one nasal swab and were asked to fill out a short questionnaire, which provided information about gender, age, current smoking habits, working area, contact with livestock outside the slaughterhouse, years of work experience in pig industry, and recent antimicrobial use. Written consent was obtained from all participants. Participants worked in various sections but primarily along the main slaughterline.

Slaughterhouse characteristics – production process. The layout of the slaughter process was similar, only differing by nuances. Upon arrival, live pigs were collected in the lairage area and only in slaughterhouse D pigs were showered with water shortly before stunning. In slaughterhouse B, all pigs were kept together in a building without heating on cement floor, without straw, water, and food for approximately 8 to 14 h. During this period pigs were stressed and stereotypic behaviour was evident. Some pigs did not react to people, but squeaked and rocked head from side to side standing against cage grill.

The animals were stunned electrically and killed by bleeding after cutting the carotid artery. In slaughterhouse D, the pigs were showered with cold water to wash evident dirt from the skin. There were two types of dehairing methods. Slaughterhouses A and C used scalding method, slaughterhouse B – singeing, slaughterhouse D – depending upon customer's request – scalding or singeing method. After scalding in warm water (65–69ºC), carcasses were dehaired, flamed, and washed with water. In the evisceration line, the carcasses were opened, organs and fat were removed, and carcasses were cut in half along the entire length of the body. Subsequently, the heads were separated from the body and the carcasses were cooled at 7 to 4ºC.

All workers wore protective equipment, including jacket, trousers, hairnet, helmet, and rubber boots, and most of the workers directly involved in the slaughter process wore disposable gloves. Slaughterhouse B was the exception, as some workers there wore overalls, and others donned their own clothes. After leaving working areas, workers washed and disinfected their hands. Workers wore their work clothing during the break. At the end of the working day, slaughterhouse, all equipment, benches, and tables were cleaned and disinfected. All workers had access to facilities to wash their hands with warm water in all slaughterhouse areas. Two slaughterhouses (A and B) had low slaughter capacity (15–20 pigs slaughtered per day) and the other two (C and D) – high slaughter capacity (100–300 pigs slaughtered per day).

Sample collection. From each slaughterhouse, several samples were collected from workers, pigs, and the environment (Table 1). One swab from each worker was taken from both nostrils. Environmental samples were collected from tables, floors, equipment (surface samples, n = 16, collected using swabs), and air (n = 8, collected using Koch's sedimentation method). All microbiological samples were stored at 4°C and the first isolation was performed within 24 h after sample collection. Nasal and rectal samples from pigs were taken after bleeding.

Table 1. Investigated samples in each slaughterhouse

Sample type	Slaughterhouses				Total
	A	B	C	D	
Nasal samples	25	15	25	35	100
Rectal samples	25	15	25	35	100
Environment	5	2	9	8	24
Carcasses	25	15	25	40	105
Workers	3	4	6	6	19

Microbiological examination. Samples from swabs were transferred on Baird-Parker Agar (Becton, Dickinson, USA) and incubated at 37°C for 24 h according to LVS EN ISO 6888-1:1999 A1:2003 "Microbiology *S. aureus* and other species - Part 1: Technique using Baird-Parker agar medium performance". 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) at 37°C for 24 h. Isolates were confirmed to be *S. aureus* by examining previous tests. Samples were categorised as 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 as positive if at least one MRSA positive colony-forming unit was isolated. These samples were categorised as MRSA-like and were stored at –20ºC until further use.

MRSA identification and *spa* typing. 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 humans were considered positive when MRSA was isolated and confirmed with multiplex-PCR from 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 slaughterhouse.

DNA was isolated by E.Z.N.A. Bacterial DNA Kit following manufacturer's instructions. DNA amount was verified 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 *mecA* genes were: *mecA* F: 5'–GTAGAA ATGACTGAACGTCCGATGA–3' and *mecA* R: 5'– CCAATTCCACATTGTTTCGGTCTAA–3'.

Amplification of DNA was performed in an Applied Biosystems 2720 thermal cycler using the following conditions: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation (94°C for 1 min), annealing (55°C for 1 min) and extension (72°C for 1 min), followed by final extension at 72°C for 10 min. The amplicons were separated using a 2% agarose gel. After electrophoresis, fragments were visualised by UV transilluminator and photographed for visual prove. *MecA* positive samples were 310 basis-pair-long. *Spa* typing was performed as described by Shopsin *et al*. (25). The *spa* gene typing was performed through the Ridom Spa server (www.spaserver.ridom.de).

Antimicrobial susceptibility testing. Randomly selected 57 MRSA positive samples from a total of 78 MRSA isolates were tested for antimicrobial susceptibility by disk diffusion method using Oxoid Antimicrobial Susceptibility Disks (Thermo Scientific, USA), following recommendations of Clinical and Laboratory Standards Institute (CLSI, USA) for inoculum preparation, inoculation, and incubation (5). The results were interpreted 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 (Co-trimoxazole) (SXT; 25 µg),

meropenem (MEM; 10 µg), and vancomycin (VA; 30 µg). After 24 h of incubation at 37ºC, inhibition zones were measured in millimetres on the Mueller-Hinton agar plates (Oxoid, UK) and interpreted according to manufacturer's directions.

Data statistical analysis. Statistical analysis was conducted using software SPSS 16 (SPSS, INC., 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 slaughterhouses and pig dehairing methods. The Chi-square test was used to analyse whether the different slaughterhouses were related to *S. aureus* and MRSA prevalence. Hypotheses of independence were 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's theorem was used to calculate probability to find staphylococcus in samples collected from infected pigs.

Results

Prevalence. In total, 78 MRSA strains were isolated from 348 samples (pigs after bleeding – 100 nasal samples, 100 rectal samples; 19 samples from slaughterhouse workers, 105 carcass samples, and 24 environmental samples) and 15 different *spa* types were identified among all MRSA isolates.

As shown in Fig. 1, microorganisms in pigs varied significantly (χ^2 P < 0.05); *S. aureus* prevalence varied in slaughterhouses from 52.0% to 89.0%, and MRSA prevalence from 8.0% to 88.6%. A tendency linking the slaughterhouse capacity and the MRSA prevalence was observed, namely the higher the capacity of a slaughterhouse (pigs slaughtered per day), the higher the prevalence of microorganisms. The lowest prevalence of staphylococcus was observed in slaughterhouse A: *S. aureus* 52.0% and MRSA 8.0%. The prevalence of *S. aureus* was similar in all slaughterhouses, except slaughterhouse D, but MRSA was more frequently found in slaughterhouses B and D.

MRSA prevalence in different slaughterhouses varied from zero to 24.0% in samples from carcasses and from 8.0% to 88.6% in samples from pigs after bleeding (Table 2). Positive environmental samples were found in slaughterhouse A (sample from scalding bath) and slaughterhouse C (slaughterhouse floor), but MRSA positive workers were detected in slaughterhouses A, C, and D. In addition, one human MRSA isolate *t1255* belonged to highly widespread antibiotic resistant types (Pen-Cef-Tetr and Pen-Tetr-Clin-Ery-Ge-Tri), which belonged to MRSA isolates of pig origin.

As seen in Fig. 2, 51% of all pigs were MRSA positive. In 17% of all pigs only nasal samples were MRSA positive, in 20% – only rectal samples, but in 14% of all pigs – both nasal and rectal samples were MRSA positive. Data analysis revealed that MRSA and *S. aureus* positive sample distribution, which depended on sample source, was similar. Taking only nasal or rectal samples for MRSA testing decreased the probability to find a microorganism to almost 17% – 20%. According to Bayes's theory, the probability to identify a pig infected with MRSA by taking rectal samples is 0.67 and by nasal samples – 0.61; the probability to detect a pig infected with *S. aureus* by taking only rectal samples is 0.69, and by only nasal samples – 0.65.
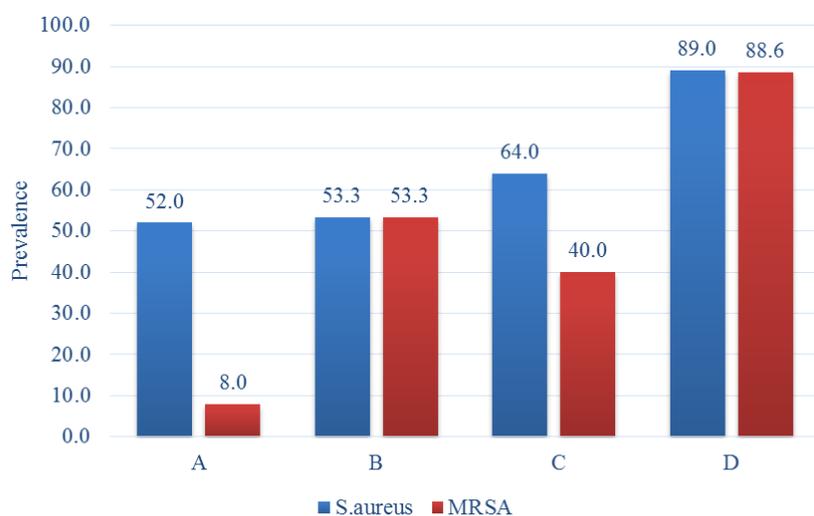

Fig. 1. Prevalence of staphylococcus in slaughterhouses

Table 2. MRSA prevalence in slaughterhouses

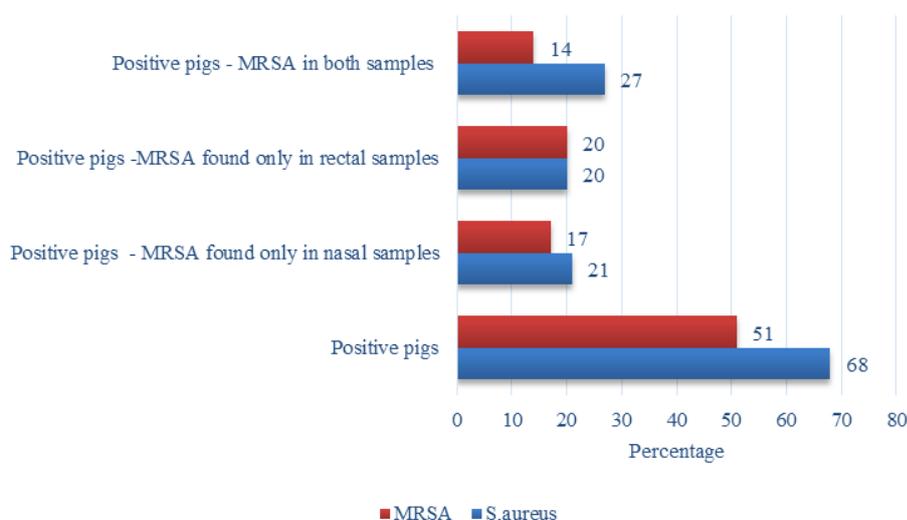| Slaughterhouse	Pigs		Carcasses		Workers		Environment	
A	2/25	8.0%	0/25	0	1/3	33.3%	1/5	20.0%
B	8/15	53.3%	0/15	0	0/4	0	0/2	0
C	10/25	40.0%	6/25	24.0%	2/6	33.3%	1/9	11.1%
D	31/35	88.6%	1/40	2.5%	1/6	16.7%	0/8	0
Total	51/100	51.0%	7/105	6.7%	4/19	21.1%	2/24	8.3

Fig. 2. *S. aureus* and MRSA isolation from pigs depending on sample type. In slaughterhouse D, two sets of different MRSA *spa* types were isolated in two animals (*spa* type *t337* together with *spa* type *t11744* in one, and *spa* new type A together with *spa* type *t11744* in the other)

In total, 57 MRSA isolates with 14 different *spa* types (Tables 3 and 4) were recovered. Table 3 shows the MRSA distribution depending on *spa* type, sample origin, and resistance to several antibiotics. The number of different *sp*a types was higher in slaughterhouses with greater pig production numbers (slaughterhouse D) and higher MRSA prevalence (slaughterhouse D). We established two *spa* types in slaughterhouse A, five in slaughterhouse C, three in slaughterhouse B, and eight in slaughterhouse D.

MRSA *spa* type *t1333* (n = 15) was found in slaughterhouses C and D, *spa* type *t011* (n = 6) was found in slaughterhouses B and C, *spa* type *t1250* (n = 2) was found in slaughterhouses C and D, whereas other MRSA *spa* types were found only in one slaughterhouse. The most widespread *sp*a types were *t1333*, *t337*, *t11744*, and *t011*. The analysis of MRSA strains revealed that *spa* types *t1774* and *t337* belonged to *ST*9, and types *t011* and *t808* to *ST398*.

Table 3. MRSA origin

Spa-type	Slaughterhouse	Antibiotype number	Antimicrobial resistance profile	Origin (n)
t011	C	4	Pen-AmCl-Cef-Tetr	Slaughtered pig (n = 1)
		6	Pen-AmCl-Cef-Tetr-Ery	Slaughtered pig (n = 1)
	B	10	Pen-Cef-Clin-Ery-Ge	Slaughtered pig (n = 1)
		26	Pen-Tetr-Clin	Slaughtered pig (n = 1)
		28	Pen-Cef-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
		30	Pen-Cef-Cip-Tetr-Ary-Ge	Slaughtered pig (n = 1)
t1333	C	1	Pen	Slaughtered pigs (n = 2), carcass (n = 1)
		7	Pen-Am-Cef-Tetr-Clin-Ery-Ge	Carcass (n = 2)
		19	Pen-Tetr-Clin-Ery-Ge	Slaughtered pig (n = 1)
		20	Pen-Tetr	Slaughtered pigs (n = 2), carcass (n = 1)
		22	Tetr-Clin-Ery	Slaughtered pig (n = 1)
		23	Pen-Cef-Tetr-Clin-Ery	Carcass (n = 1)
		25	Pen-Tetr-Clin-Ery	Slaughtered pig (n = 1)
		28	Pen-Cef-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
	D	20	Pen-Tetr	Slaughtered pig (n = 1)
		19	Pen-Cef-Clin-Ery-Ge	Slaughtered pig (n = 1)
t337	D	14	Pen-Cip-Tetr-Ery-Ge-Tri	Slaughtered pig (n = 1)
		15	Pen-Cip-Trtr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
		17	Pen-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 3)
		18	Pen-Tetr-Clin-Ery-Tri	Slaughtered pig (n = 2)
		19	Pen-Tetr-Clin-Ery-Ge	Slaughtered pig (n = 2)
		20	Pen-Tetr	Slaughtered pig (2)
		1	Pen	Slaughtered pig (n = 1)
t808	D	17	Pen-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
t2451	C	1	Pen	Worker at slaughterhouse (n = 1)
t899	A	1	Pen	Slaughtered pig (n = 1)
		5	Peb-AmCl-Tetr-Tri-Me	Slaughtered pig (n = 1)
t11744	D	10	Pen-Cef-Clin-Ery-Ge	Slaughtered pig (n = 2)
		17	Pen-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
		18	Pen-Tetr-Clin-Ery-Tri	Slaughtered pig (n = 1)
		19	Pen-Tetr-Clin-Ery-Ge	Slaughtered pig (n = 1)
		28	Pen-Cef-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
		29	Pen-Cef-Tetr-Clin-Ery-Ge-Tri-Me	Slaughtered pig (n = 1)
t127	C	11	Pen-Cef-Tetr	Carcass (n = 1)
		20	Pen-Tetr	Slaughterhouse floor (n = 1)
t1250	D	17	Pen-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
	B	20	Pen-Tetr	Slaughtered pig (n = 1)
t2421	D	17	Pen-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
		26	Pen-Tetr-Clin	Slaughtered pig (n = 1)
t1255	C	17	Pen-Tetr-Clin-Ery-Ge-Tri	Slaughterhouse worker (n = 1)
t318	B	28	Pen-Cef-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 2)
		29	Pen-Cef-Tetr-Clin-Ery-Ge-Tri-Me	Slaughtered pig (n = 1)
t421	D	28	Pen-Cef-Tetr-Clin-Ery-Ge-Tri	Slaughtered pig (n = 1)
new A	D	2	Pen-AmCl-Tetr	Slaughterhouse worker (n = 1)
new B	A	27	Pen-Cef-Tetr-Clin-Ery-Ge	Slaughterhouse worker (n = 1)

* Pen – penicillin, AmCl – amoxicillin with clavulanic acid, Cef – cephalexin, Cip – ciprofloxacin, Clin – clindamycine, Ery – erythromycin, Ge – gentamycin, Me – meropenem, Tetr – tetracycline, Tri – trimethoprim/sulphamethoxazole, Van – vancomycin

Table 4. MRSA *spa* type distribution in slaughterhouses

Slaughterhouse	*Spa* types
A	*t899* (n = 2), new B (n = 1)
C	*t1333* (n = 13), *t011* (n = 2), *t2451* (n = 1), *t127* (n = 2), *t1255* (n = 1)
B	*t01*1 (n = 4), *t1250* (n = 1), *t318* (n = 3)
D	*t1333* (n = 2*)*, *t337* (n = 12), *t808* (n = 1), *t11744* (n = 7), *t1250* (n = 1), *t2421* (n = 2), *t421* (n = 1), newA (n = 1)

Antimicrobial susceptibility testing of the selected MRSA isolates revealed the presence of 22 different antibiotic resistance profiles (ARPs). The distribution of MRSA *spa* type among the samples is shown in Table 5 and Figs 3 and 4.

The present study demonstrated that 26.3% of MRSA isolates belonged to *spa* type *t1333* (n = 15), and other most common *spa* types were *t337* (n = 12) and *t11744* (n = 7). Two to nine different ARP were detected among one MRSA *spa* type (Table 5). The most frequently occurring *spa* types showed the highest antibiotic resistance heterogeneity, for example *spa* type *t1333* belonged to nine ARPs (see Table 5), while the lowest heterogeneity was evident among MRSA *spa* type *t318* (n = 2).

MRSA isolate *t1255* that originated from a slaughterhouse worker also belonged to MRSA isolates from pig origin and had a highly widespread ARP Pen-Tetr-Clin-Ery-Ge-Tri. In total, 53% of the isolates belonged to five most prevalent ARPs shown in Table 6.

Two most common ARPs (28.0% of all MRSA isolates) belonged to eight *spa* types. MRSA *spa* type *t1333* was evident in ARP Pen-Tetr, Pen-Cef-Tetr-Clin-Ery-Ge-Tri, Pen, and Pen-Tetr-Clin-Ery-Ge, but *spa* type *t11744* was evident in ARP Pen-Tetr-Clin-Ery-Ge-Tri, Pen-Cef-Tetr-Clin-Ery-Ge-Tri, and Pen-Tetr-Clin-Ery-Ge. Nearly all MRSA isolates showed multidrug resistance.

The highest antimicrobial resistance was seen to five antibiotics – 24.6% of all MRSA isolates, six antibiotics – 19.3% of all MRSA isolates, and seven antibiotics – 15.8% of all MRSA isolates. Fewer isolates exhibited resistance to two (14.0%), one (10.5%), and three (8.8%) antibiotics.

The 98.2% of all MRSA isolates were resistant to penicillin, 89.1% to tetracycline, 69.1% to erythromycin, 65.5% to clindamycin, 50.9% to gentamycin, 40.0% to trimethoprim/ sulphomethazole, 29.1% to cephalexin, 10.9% to amoxicillin combined with clavulanic acid, 9.1% to ciprofloxacin, and 3.6% to meropenem.

All isolates were sensitive to vancomycin, except six, which showed intermediate sensitivity to vancomycin: *t011* (n = 1), *t2337* (n = 2), *t11744* (n = 2), *t2421* (n = 1).

Comparing MRSA *spa* type antibiotic resistance, it was found that even within the same *spa* type there were different ARPs, therefore the average percentage resistance was calculated from all MRSA isolates in one *spa* type. The results are shown in Fig. 4.

All five most common *spa* types in our samples differed from each other, but a shared tendency was seen, indicating that among all these types, almost 80% to 100% were resistant to tetracycline and penicillin. *Spa* type *t1333* was the most widespread from all *spa* types, but it had lower antimicrobial resistance in some samples showing resistance to seven antibiotics, while *spa* type *t318*, which was the second most spread MRSA *spa* type, had the highest antimicrobial resistance among all MRSA *spa* types showing antimicrobial resistance to eight and seven antibiotics. Among all most common MRSA *spa* types, except *t899*, the majority was resistant to seven types of antibiotics (penicillin, tetracycline, cephalexin, clindamycin, erythromycin, gentamycin, trimethoprim/ sulphamethoxazole). MRSA resistance was observed even in the case of antibiotics which are not frequently used or are prohibited for use in food chain animal treatment, such as gentamicin and cephalexin. In some rare cases MRSA isolates showed resistance and intermediate sensitivity to meropenem and vancomycin (not shown in figures).

Table 5. Characterisation of the most frequently occurring MRSA *spa* types

Spa type	All MRSA isolates (%)	The highest resistance to current antibiotics	Total amount of different antimicrobial resistance profiles
t318	5.3	Cef, Gen, Ery, Clin, Tetr, Pen	2
t011	10.5	Pen, Tetr, Cef, Am-Clav	5
t11744	12.3	Ery, Clin, Tetr, Pen, Gen	6
t337	21.0	Pen, Tetr, Ery, Clin	7
t1333	26.3	Pen, Tetr, Ery, Clin	9

Table 6. Characterisation of most frequently found antibiotic resistance profiles (ARPs)

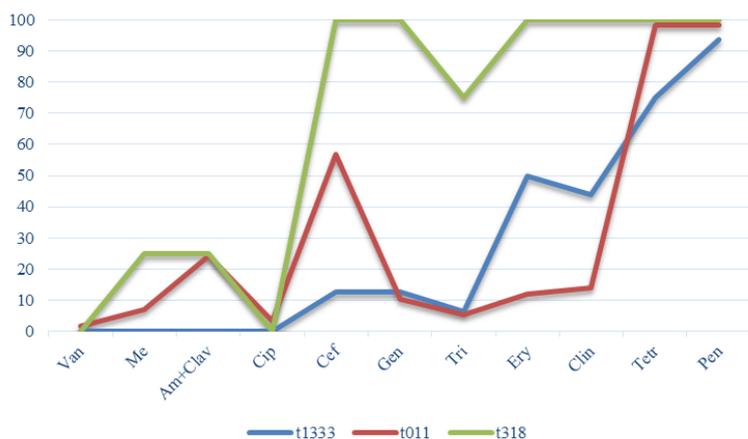| Most frequently found ARPs	Number of MRSA isolates	All MRSA isolates (%) (n = 57)	Number of different MRSA *spa* types
Pen-Tetr-Clin-Ery-Ge-Tri	8	14.0	6 (*t337, t808, t11744, t1250, t2421, t1255*)
Pen-Tetr	8	14.0	4 (*t1333, t337, t127, t1250*)
Pen-Cef-Tetr-Clin-Ery-Ge-Tri	6	10.5	5 (*t011, t1333, t11744, t318, t421*)
Pen	4	7.0	4 (*t1333, t337, t2451, t899*)
Pen-Tetr-Clin-Ery-Ge	4	7.0	3 (*t1333, t337, t11744*)

Fig. 3. Antimicrobial resistance of MRSA most common *spa* types: *t1333*, *t011*, and *t318*

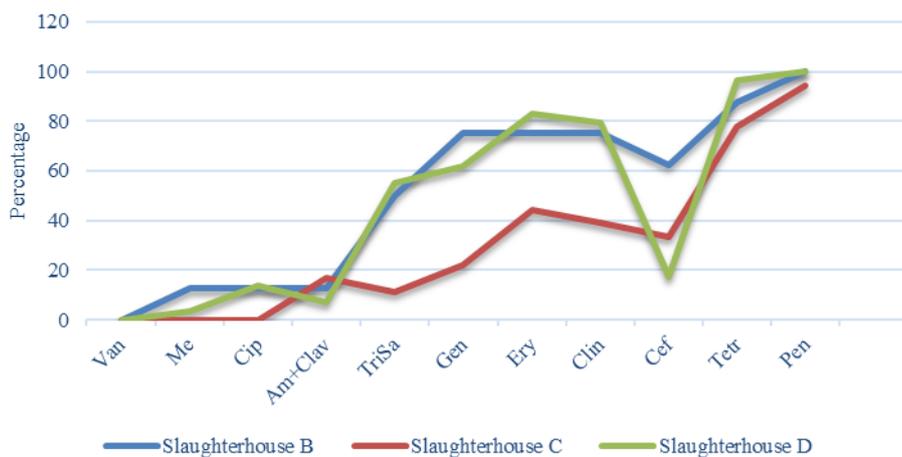**Fig. 4.** Antimicrobial resistance of MRSA most common *spa* types: *t1744* and *t337*

Fig. 5. Antimicrobial resistance of MRSA most common *spa* types by slaughterhouse

Comparing antibiotic resistance in slaughterhouses (Fig. 5), greater antibiotic resistance appeared to trimethoprim/sulphamethoxazole, gentamycin, and clindamycin in slaughterhouses B and D, but in slaughterhouse C there was higher resistance to cephalexin. In slaughterhouse A (not shown in figure) three MRSA isolates were resistant to penicillin and two isolates were resistant to tetracycline. Samples

from slaughterhouse A (one sample for each antibiotic) showed resistance to amoxicillin combined with clavulanic acid, trimethoprim/sulphamethoxazole, meropenem, cephalexin, clindamycin, erythromycin, and gentamycin. However, there was no evident difference in antibiotic resistance among slaughterhouses in relation to slaughter capacity.

The analysis of dehairing methods (singeing and scalding) revealed that there was no statistically significant difference (according to Fishers' test P > 0.05) between both methods regarding the influence on microorganism distribution on carcasses. *S. aureus* was isolated from carcasses treated with scalding method in 9.9% (n = 7) and MRSA in 7% (n = 5), while in the case of singeing method *S. aureus* was isolated in 2.9% (n = 1) and no MRSA isolates were detected.

Discussion

This study investigated transmission and distribution of *S. aureus* and MRSA in individual pigs, slaughterhouse environment, and slaughterhouse workers. Significant findings included the identification and detailed characterisation of MRSA isolates from Latvian pigs, which was done for the first time. Furthermore, our study demonstrated MRSA colonisation status between pigs and carcasses. In addition, we evaluated the influence of the two types of pig dehairing methods in slaughterhouses with reference to their role in *S. aureus* prevalence, MRSA distribution, and antibiotic resistance level tendencies among different pig slaughterhouses depending on production efficiency. Presented research revealed that MRSA distribution and ARP depended on MRSA *spa* types. Antimicrobial contamination is one of the main factors in the emergence and transmission of MRSA in pigs and requires further investigation. The main reason for the contamination is close contact during the transportation or slaughterhouse procedures in confined spaces, which is consistent with the suggestions by Moodley *et al.* (17) and Broens *et al.* (3) that the likely source of MRSA transmission between farm pigs was the direct contact with their snout, skin, vagina, and faeces. Broens *et al.* (3) found that as many as 60% of previously MRSA negative pigs were positive at the stunning after transportation and holding (total time 3.75–16.5 h) in slaughterhouse, after close contact with other pigs from different farms. Another, though less likely, source of MRSA for pigs in lorries and lairages may be the lorry drivers and/or abattoir personnel (3). Studies in pig slaughterhouses have shown MRSA prevalence of 5.6% among slaughterhouse workers and 22% among lorry drivers (28).

In addition, ARP, microarray, and MRSA *spa* types found in this study strongly support the view that in slaughterhouses with higher pork production the antimicrobial resistance is higher, and there is a greater MRSA *spa* type heterogeneity. However, it was demonstrated that many factors, in addition to pig colonisation status in slaughterhouses, are likely to influence the carrier status. Such factors might include antibiotics used for treatment and prophylaxis, animal welfare condition, pig population density, and contact among them.

In our study MRSA was found only in two environmental samples (surface sample from slaughterhouse floor and a sample from scalding bath) and one *S. aureus* isolate from slaughterhouse A (from scalding bath), while other investigations have shown MRSA distribution in barn spaces. In Germany (11) MRSA prevalence was evident in 23 out of 27 investigated pig barns (85.2%) and the prevalence in dust samples appeared to be 100%; conversely, according to EFSA report (10) the prevalence in dust samples is 0%. In previously mentioned studies testing dust and farm air, filtration methods and specific equipment (impingers, a six-stage Andersen air sampler) were used, but in our study Koch's sedimentation method was used, and it could be the reason for such a low MRSA detection level; however, it does not explain low MRSA prevalence in surface samples taken from slaughterhouses (tables, knives, gloves, floor, and pork boxes). This failure to detect airborne MRSA and *S. aureus* in slaughterhouses by means of Koch's sedimentation method did not guarantee the absence of these bacteria in the air. Dust is probably the main factor for MRSA distribution in the air since some surface and staff samples were also positive. MRSA evidence in the environment (air and surface samples) reveals the difficulties regarding the reduction of bacterial spread within the slaughterhouse.

Slaughterhouses should be strictly controlled to avoid contamination of healthy animals, carcasses, and workers by infected animals. Possible control measures could include the avoidance of pig contacts from different farms and new group forming, as well as effective vehicle and environment cleaning and disinfection and reducing antimicrobial use, especially for prophylactic purposes.

Burns *et al.* (4) found that more than 1/3 of *S. aureus* isolates were resistant to tetracycline and erythromycin. Similar situation was observed in our study, where the resistance to tetracycline was 89.1% and to erythromycin 69.1%. Moreover, 50.9% of isolates were resistant to gentamycin and 29.1% to cephalexin, which were not used for pig treatment in farms, but resistance to penicillin, which was used as the first choice antibiotic several years ago, and to tetracycline, which is administrated in high amounts in Latvia, reached 98.2% and 89.1%.

Data similar to our study were present in Italian research (20) concerning antibiotic resistance profiles, where most MRSA isolates were multidrug-resistant, including resistance to clindamycin, tetracycline, and erythromycin. Study from Denmark (32) showed that *spa* type *t034* was most widespread, whereas in studies

of Tenhagen *et al.* (27), Broens *et al.* (3), Crombe *et al.* (6), Friese *et al.* (11), Pletinckx *et al.* (23), and in our study *spa* type *t1333*, isolated from two slaughterhouses, was most frequently found. Other most widespread *spa* types were *t33*7, *t11744*, and *t011*. MRSA *t011 sp*a type was found in poultry farms in Belgium (19), Denmark (1), and Italy (20). *Spa* type *t337* was found by Normanno *et al.* (20). According to Fisher's test value ($1.1*10^{-11}$), statistically significant differences were found regarding the origin of the samples and MRSA *spa* types isolated from slaughterhouses. *Spa* type *t1333* was also one of the most frequently found MRSA *spa* types in the study by Tenhagen *et al.* (27) and it was also isolated together with *t127* by Agerso *et al.* (1). The results of our study are in agreement with the findings by Pletinckx *et al.* (23), who demonstrated different antimicrobial resistance profiles per *spa* type.

Human infections caused by MRSA are one of the main causes of morbidity and mortality in industrialised countries. In addition to the traditional routes of MRSA infection, it has recently been demonstrated that the direct transmission to humans takes place *via* contact with farm animals (31). Also, it is suggested that infection risk is created by handling of animal food products, for example raw meet (10).

Many studies on the prevalence of MRSA in farm animals and their carcasses have included pigs, which are currently the most important reservoir of MRSA (22). There are numerous reports on the prevalence of MRSA in various kinds of meats, especially in pork from Japan, Korea, Europe, and USA (21). The prevalence of MRSA in pigs and pig carcasses for human consumption is a public health issue, and the emergence of MRSA infections in humans has been well documented in several European countries (32). In several studies performed in slaughterhouses, MRSA prevalence in live pigs varied; *e.g.* in Dutch abattoirs 39% pigs and 81% slaughter batches were MRSA positive (8).

Our findings are in agreement with those reported by other researchers (20) and indicate that the risk of foodborne toxic infection linked to consumption of MRSA-contaminated meat is quite limited. Our study showed that during the slaughter process when high hygiene rules are applied, MRSA contamination decreased to zero in carcasses in relation to infected live pigs (prevalence 30% to 65%). The high presence of MRSA in pigs is a potential professional hazard for staff working in the meat production chain (workers in farms and slaughterhouses, carriers, and veterinarians). It is known that people working several hours a week in direct contact with pigs infected with MRSA are exposed to high risk of nasal colonisation (9, 17, 31). The general population shows a high prevalence (approximately 30%) of *S. aureus* nasal colonisation, whereas MRSA nasal colonisation levels are lower (0.7%−1.5%) depending on the geographical area (18). Human colonisation implies that carriers become

a staphylococcal reservoir and may transfer the infection to others, or contaminate foods and food surfaces during handling. In addition, subclinical carriage of MRSA by humans is considered a risk factor for subsequent occurrence of clinical disease (15). MRSA from food-producing animals can be transferred to the slaughterhouse environment, and thus constitute a source of contamination for slaughterhouse personnel (31). In our survey, the anterior nares of 4 out of 19 investigated workers from pig slaughterhouses were colonised by MRSA, which is a substantially higher proportion when compared to other studies: in Italy 9 out of 113 (21), in Switzerland no MRSA in 179 slaughterhouse workers (14), in China no MRSA in 107 slaughterhouse workers (7), and in the Netherlands 5.6% of nasal carriage in 195 pig slaughterhouse workers (29). Similar results were reported by Gilbert *et al.* (12) in the Netherlands, who found that 11 of 341 pig slaughterhouse workers (3.2%) were nasal carriers of LA-MRSA. In a Spanish study 14.3% of pig slaughterhouse workers were nasal MRSA carriers (16). High contamination with MRSA in barn or slaughterhouse may also be an issue of occupational health. It has been proven in several studies that humans working in pig farms and slaughterhouses carry MRSA of the same sequence type as swine (30). The presence of MRSA and *S. aureus* in the environment, carcasses, and worker nares is not surprising since the staff do not wear any respiratory masks and are in close physical contact with pigs. Moreover, their hands, gloves, equipment, clothes, and boots can serve as an infection transmitters and contaminants.

Antimicrobial resistance in human bacterial pathogens and zoonotic agents is growing worldwide, and this may threaten effective treatment of infections in humans. Multidrug-resistance was evident in our MRSA isolates. Nearly all isolates displayed resistance to two or more classes of antimicrobials and some of them were resistant or displayed intermediate sensitivity to vancomycin and meropenem. This indicates the development of resistance to previously mentioned antibiotics. These findings are in agreement with other studies on high MRSA prevalence and antimicrobial resistance in isolates from pigs, pork, and humans (2). As expected, 98.2% of isolates were resistant to penicillin and 89.1% to tetracycline, but quite a high resistance appeared to erythromycin (69.1%), clindamycin (65.5%), gentamycin (50.9%), and trimethoprim/sulphamethoxazole (40.0%). Several studies have shown similar resistance (2, 5). Pletinckx *et al.* (23) found high resistance to trimethoprim, lincomycin, and ciprofloxacin. Moreover, comparing the study by Tenhagen *et al.* (27) with our findings, it can be noted that antimicrobial resistance of MRSA *t1333 spa* type has increased. Tenhagen *et al.* (27) demonstrated that the most widespread *spa* type *t1333* was susceptible to methicillin, but in our study *spa* type *t1333* showed high resistance to clindamycin (43%),

erythromycin (50%), tetracycline (75%), and penicillin (93%).

Finally, it is known that MRSA prevalence and *spa* types differ depending on the slaughter capacity and good hygiene practice. The pigs used in our study originated from different slaughterhouses in our country, and this could be the reason for the wide heterogeneity of the MRSA *spa* types.

The presence of MRSA and *S. aureus* in carcasses intended for human consumption is a potential health hazard and it must be controlled by implementing personnel training on issues of biosecurity and food hygiene, throughout the meat chain from primary production to market. Another equally important fact is quite high MRSA occurrence among slaughterhouse workers, which also increases pork contamination risk. As far as we know, this is the first report documenting the prevalence and characteristics of MRSA in slaughterhouses and staff involved in pig industry in Latvia.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Animal Rights Statement: According to the Directive 2010/63/EU of the European Parliament and Council of 22 September 2010 on the protection of animals used for scientific purposes, there was no need for specific permission from competent authority, because the samples were obtained during the slaughter process after pig stunning and bleeding.

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