INVESTIGATION OF MRSA ISOLATES FROM PIG ORGIN, ENVIRONEMNT AND STUFF BY *SPA* TYPING, ANTIMIROBIAL RESISTANCE AND SE GENE DETECTION

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Abstract. MRSA (methicillin resistant *Staphylococcus aureus*) is hard curable and highly resistant zoonotic soft tissue pathogen which is also capable to produce enterotoxins and cause food toxiconfections. The aim of the study was the characterization of MRSA isolates from different pig farms and slaughterhouses based on interaction of antimicrobial resistance, *spa* type, SE genes and source of MRSA isolates. In total 405 pigs, 105 carcasses, 34 workers as well as 46 samples from environment from several farms and slaughterhouses were examined by conventional microbial and molecular methods. In total 155 (14.6%) MRSA isolates were detected from 1064 samples tested and included 11 positive pigs (27.4%) form all tested pigs, 2 isolates (4.3%) from environment, 3 isolates (4.3%) from milk, 7isolates (6.7%) from pig carcasses and 7 isolates (20.6%) from workers.

From all MRSA isolates 48.4% contained *sea*, 1.3% *seb*, 5.9% *sec* and 9.7% *seh*. Study showed that *sea* mostly appears alone (70.4%) in MRSA isolates or together with *seh* (14.8%), but *seh* trends to be together in isolate genome with other genes that are coding SE. In the same time in these MRSA isolates *sec* appears alone or together with *sea*. Contrary to other investigations, our study indicated high *sea* distribution in MRSA isolates and only some isolates with *sec*, moreover among MRSA *spa* types with high *sea* distribution antimicrobial resistance was lower.

Keywords: methicillin resistant *Staphylococcus aureus*, pigs, zonosis, enterotoxins genes

Introduction. *Staphylococcus aureus* is a well-known commensal pathogen of large number of animal species, including humans. A wide variety of infections can be caused by *S. aureus*, from superficial (skin and tissue infections) to life-threatening septicaemia (Armand-Lefevre et al. 2005, Graveland et al. 2010). A spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in livestock is a serious public health threat (Graveland et al. 2011, Cunny et al. 2013). The rapid spread of MRSA in pigs and farm animals worldwide has raised major public health (Verkade and Kluytmans 2014). Among food animals, pigs have been implicated as one source of potential infections. Colonised animals may act as a MRSA reservoir not only for livestock, but also for humans with close contact to animals, farmers and veterinarians (Voss et al. 2005, Huijsdens et al. 2006, Wulf et al. 2008, Wettstein Rosenkranz et al. 2014). In Europe, in survey using standardized sampling and testing methods the prevalence per country in holdings with breeding pigs ranged from 0% to 51.2% in 2008 (EFSA, 2009). When MRSA carrying animals are slaughtered, MRSA may contaminate carcasses, the environment and spread to abattoir workers. In addition, if carcasses and meat are contaminated, MRSA can enter the human food chain (Kluytmans et al. 2010).

MRSA produce the notable virulence factors, staphylococcal enterotoxins (SE) encoded by SE genes and is worldwide the most important pathogen in foodborne intoxication. *S. aureus* causes gastrointestinal symptoms like nausea, emesis, abdominal cramps and diarrhoea. Mostly five classical SE (SEA-SEE) have been recognized and sporadic cases as well outbreaks due to these enterotoxins are described (Nitzsche et al. 2007, Argudin et al. 2010, Chao et al. 2014) – raw meat and meat products including fermented products such as sucuk (Guven et al. 2010). SEA, SEB, SEC and SEH enterotoxins have emetic activity (Argudin et al. 2010) and are associated with food poisoning. SEA is most common in food poisoning and outbreaks, SEC is in the first place from detected enterotoxins from animal origin, but SEB is even studied as a biological weapon (Pinchuk et al. 2010). Enterotoxin SEH is one of the latest discovered enterotoxins of *S. aureus* and still investigation is going on in this field.

Antimicrobial resistance is also an important issue for public health worldwide. The development of resistance both in human and animal bacterial pathogens has been associated with the extensive therapeutic use of antimicrobials or with administration as growth promoters in food animal production (Barber et al. 2003). MRSA is a major concern worldwide due to its resistance to different types of antibiotic including beta-lactam antimicrobials (David and Daum 2010). A study from Louisiana found that bacteria in different types of meat were commonly resistant to penicillin, ampicillin, tetracycline, erythromycin, clindamycin, levofloxacin and ciprofloxacin from 13% to 71% depending from antibiotic (Pu et al. 2011).

The aim of the study was the characterization of MRSA isolates from different pig farms and

slaughterhouses based on interaction of antimicrobial resistance, *spa* type and source of isolates.

Material and Methods

Farms and slaughterhouses

During this study three Latvian pig farms and four slaughterhouses were sampled from October to March. These farms and slaughterhouses were selected with different size, pig slaughter capacity and were located in different areas of Latvia. Herd size varied from 250 to 2000 sows and 1500 to 12000 fattening pigs, but slaughter capacity varied from 15 to 300 slaughtered pigs per day.

Sample collection

From each farm and slaughterhouse were taken several samples from workers, pigs and environment (see Table 1). One sample from each worker was taken from both nares. Environmental samples were obtained in separate work areas air and equipment. All microbiological samples were stored in +4 °C and first isolation was made during 24 hours after sample collection.

Table 1. **The number of investigated samples from pigs, carcasses, workers and environment**

Sample type	Slaughterhouses and farms							Total samples
	f-A*	f-B	f-C	sl-D*	sl-E	sl-F	sl-G	
Nasal	96	105	104	25	15	25	35	405
Rectal	96	105	104	25	15	25	35	405
Environment	5	9	8	5	2	9	8	46
Milk	18	25	26	0	0	0	0	69
Carcasses	0	0	0	25	15	25	40	105
Workers	4	4	7	3	4	6	6	34
*f - farms; sl - slaughterhouses								

Microbiological examination. Samples from swabs were transferred on Baird-Parker Agar (Becton, Dickinson, USA) and incubated in 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 Mannitol Salt agar plates were determined as *S. aureus*-like and were inoculated on CHROMagar Staph aureus plate (Becton Dickinson, USA) in 37 °C for 24 hours. 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 ^{0}C until further use.

MRSA identificationand *spa* typing.

MRSA identification and further examination was performed at Riga Stradins University and at Biotechnology Research Laboratory of Latvia University of Agriculture. 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 PCR form at least one anatomical sampling site. The dominant pig *spa* and *SCCmec*-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 (OMEGA BIO-TEK E.Z.N.A.) 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'–GTAGAAATGACTGAACGTCCGATGA–3' and *mecA* 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 by a 2% agarose gel. After electrophoresis fragments were checked out by UV transilluminator visualization and photographed for visual prove. *mecA* positive samples were 310 bp long.

Spa typing was performed as has been described (Shopsin et al., 1999). The *spa* gene typing was performed through the Rindom Spa server (www.spaserver.ridom.de).

Detection of enterotoxin production

Polymerase chain reaction (PCR) was performed by HotStarTaq® Plus Master Mix Kit Qiagen, DE following manufacturer's instructions. The primer sequences for the *sea* genes were: SEA - F: 5' – TTGGAAACGGTTAAAACGAA – 3' and sea R: 5' – GAACCTTCCCATCAAAAACA – 3'. The primer sequences for the *sec* genes were: sec - F: 5' – GACATAAAAGCTAGGAATTT – 3' and SEC R: 5' – AAATCGGATTAACATTATCC – 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 (50 °C for 1 min (*sea, seb*)) and (47°C for 1 min (*sec, seh*)) (47°C for 1 min (*sec*)) extension (72 °C for 1 min), following final extension at 72 °C for 10 minutes.

The amplicons were separated by a 2% agarose gel. After electrophoresis fragments were checked out by UV transilluminator visualization and photographed for visual prove. *sea* positive samples were 120 bp long, *seb* – 478 bp long, *sec* 257 bp long and *seh* – 360 bp long.

Antimicrobial susceptibility testing.

Randomly selected 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 (Co-trimoxazole) (SXT; 25µg), Meropenem (MEM; 10 µg), Vancomycin (VA; 30 µg). After 24 h of incubation at 37 ^{0}C, inhibition zones were measured on the Mueller-Hinton agar plates (Oxoid, UK) and interpreted according to the manufacturer directions.

Data statistical analysis

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 slaughterhouses and farms. The Chi-square test was used to analyse whether the different farms and slaughterhouses were related to *S. aureus,* MRSA and SE prevalence. Hypothesis 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 significance has determined. 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.

Results. In total 155 MRSA strains from total 1064 samples (pigs nasal samples n=405, rectal samples n=405; milk n=69; samples from workers n=34, carcasses n=105, environment n=46) were isolated. In 75 (48.4%) isolates from 155 containing *sea* gene, 2 (1.3%) *seb*, 9 (5.9%) *sec* and 15 (9.7%) *seh* gene were detected.

In the present study MRSA prevalence, varied significantly (χ^2 p value <0.05) (Table 2). MRSA prevalence in pigs ranged from 4.2% to 88.6% with average value 27.4% between pigs while prevalence from carcasses were quite low – 6.7%. The highest prevalence of MRSA was observed in samples taken from slaughterhouses (8% - 88.6%; average – 51.0%), but average prevalence in farms was 19.7%. Prevalence between workers in farms and slaughterhouses was quite similar and in total 20.6% of all workers turned out to be MRSA carriers. MRSA was evident in two environment samples taken from slaughterhouses – slaughterhouse D (sample from scalding bath) and slaughterhouse F (sample from floor in evisceration place).

Table 2. **MRSA and SE gene prevalence in farms, slaughterhouses, workers and environment**

Sample type	Slaughterhouses and farms														Total	
	fA*		fB		fC		slD*		slE		slF		slG			
Pigs**	4/96	4.2%	10/105	9.5%	46/104	44.2 %	2/25	8.0%	8/15	53.3 %	10/25	40.0 %	31/35	88.6 %	111/405	27.4 %
Environment	0/5		0/9		0/8		1/5	20.0%	0/2		1/9	11.1 %	0/8		2/46	4.3%
Milk	0/18		1/25	4.0%	2/26	7.7%	0								3/69	4.3%
Carcasses	0						0/25		0/15		6/25	24.0 %	1/40	2.5%	7/105	6.7%
Workers	2/4	50.0%	1/4	25.0 %	0/7		1/3	33.3%	0/4		2/6	33.3	1/6	16.7	7/34	20.6 %
sea in pigs***	4/4	100.0 %	7/10	70.0 %	23/46	50.0 %	2/2	100.0 %	0/8	0	7/10	70.0 %	13/31	41.9 %	56/111	50.4 %
sec in pigs	0/4	0	3/10	30.0 %	2/46	4.3%	0/2	0	0/8	0	1/10	10.0 %	2/31	6.5%	8/111	7.2%
seh in pigs	1/4	25.0%	1/10	10.0 %	0		0		0		7/10	70.0 %	3/31	9.7%	12/111	10.8

*f - arms; sl– slaughterhouses
** MRSA from total investigated samples in each group
*** *sea* and *sec* % from all MRSA positive pigs, including positive milk samples (n=116)

The antimicrobial resistance of MRSA isolates differed. The highest resistance among MRSA isolates was seen against penicillin 98.4% (n=130) and tetracycline 93.9% (n=124), than followed by cephalexin – 39.4% (n=52), clindamycin– 34.0% (n=45), erythromycin – 33.3% (n=44), gentamicin – 23.5%

(n=31), trimethoprim/sulphamethoxazole – 20.5 (n=27), amoxicillin with clavulanic acid – 15.9% (n=21), meropenem – 7.6% (n=10) and ciprofloxacin – 4.5% (n=6).

In the present study gene *sea* and *sec* prevalence varied significantly ($\chi 2$ p value <0.05), *sea* - from none to 100% and *sec* from none to 30.0% depending from farm or slaughterhouse (see Figure 1), but gene *seh* prevalence varied from none in farm C and slaughterhouse D to 70.0 % in slaughterhouse F. The highest prevalence of *sea* was seen in MRSA isolates from farm A (100%) and farm B (70.0%), and slaughterhouse D (100%), and F (70.0%). Although MRSA prevalence was one of lowest – 4% and 9.5% except slaughterhouse F. *sec* gene was evident in pigs from farm B and C and in pigs from slaughterhouses F and G, but *seh* gene was evident in farms A and B, and slaughterhouse F and G.

f – farms, sl – slaughterhouses

Fig. 1. **MRSA and SE gene prevalence in pigs**

SE genes were found (see Table 3) in sample taken from environment – *sea, seb and seh* together in sample taken from slaughterhouse F floor, in human samples - *sea* together with *seh* (n=1) and *sea* in slaughterhouse F (n=1), farm B (n=1) and farm C (n=1), in carcasses in slaughterhouse F – *sea* (n=3), *sea, seb, sec* and *seh* together (n=1), in sow milk – *sea* together with *sec* in farm B (n=1) and *sec* in farm C (n=1).

The study showed that *sea* gene mostly appears alone (70.4% from all isolates that contain enterotoxin genes) in MRSA isolates or together with gene *seh* (14.8%) (see Table 3), but gene *seh* trends to be together in isolate genome with other genes that are coding SE. Gene *sec* appeared alone as well as together with gene *sea*.

Table 3. **SE gene distribution and combinations**

SE gene combination	n	Farms			Slaughterhouses				Sample (n) origin			
		A	B	C	D	E	F	G	Pigs	Carcasses	Workers	Environment
sea	57/70.4%	3	6	27	2	-	4	15	51	3	3	-
sec	5/6.2%	-	1	2	-	-	-	2	5	-	-	-
seh	1/1.2%	-	-	-	-	-	1	-	-	1	-	-
sea, sec	4/4.9%	-	2	-	-	-	1	1	4	-	-	-
sea, seh	12/14.8%	1	1	-	-	-	7	3	11	-	1	-
sea, seb, seh	1/1.2%	-	-	-	-	-	1	-	-	-	-	1
sea, seb, sec, seh	1/1.2%	-	-	-	-	-	1	-	-	1	-	-

The prevalence of *sea* gene in MRSA varied from 30.0% in fattening pigs to 60.9% in 4 – 4.5 month old piglets, while *sec* prevalence ranged from none in fattening pigs to 13.0% in 4 – 4.5month old piglet groups. As it is seen from Figure 2 *sea* and *sec* prevalence inclines to increase and decrease in several age groups in similar way not making huge differences as well as MRSA prevalence except suckling piglet group, while *seh* gene was evident in pigs shortly before slaughter (slaughterhouse F 70.0%) and in farm A in 4 – 4.5 month old pigs and farm B in fattening pigs (n=1).

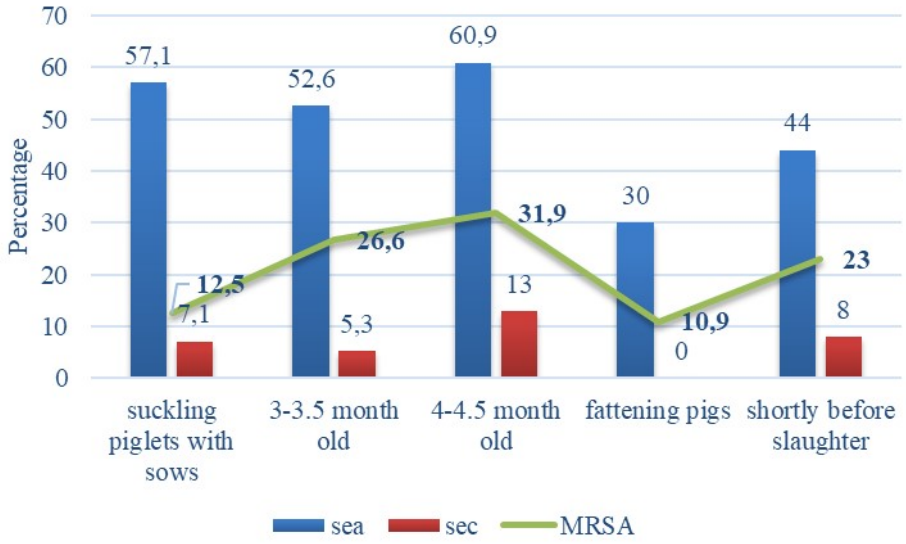


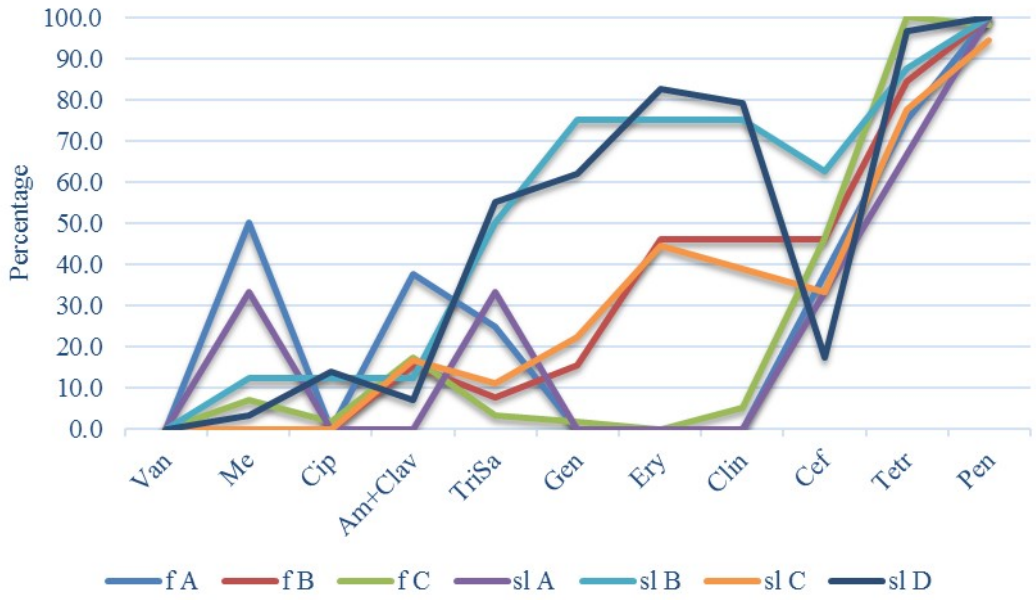
Fig. 2. **SE gene and MRSA prevalence in different pig age groups**

Table 4. **MRSA SE genes within *spa* types**

Spa type	Total isolated	*sea*		*sec*		*seh*	
		%	n	%	n	%	n
t127	2	100	1	50	1	100	2
t318	3	-	-	-	-	-	-
t337	12	58.3	7	8.3	1	24.9	3
t400	4	100	4	50	2	-	-
t693	1	100	1	-	-	-	-
t808	6	66.7	4	0	0	0	0
t899	5	100	5	0	0	0	0
t1255	2	100	2	-	-	-	-
t1250	2	-	-	-	-	-	-
t1333	18	66.7	12	11.11	2	33.3	6
t1580	1	-	-	-	-	-	-
t1985	4	50	2	-	-	-	-
t421	1	0	-	-	-	-	-
t2421	2	100	2	-	-	-	-
t2383	1	-	-	-	-	-	-
t2451	1	100	1	-	-	100	1
t11744	7	42.9	3	143	1	-	-
t011	57	54.4	31	-	-	-	-
t1580	1	-	-	-	-	-	-
new A	1	100	1	-	-	-	-
new B	1	-	-	-	-	-	-

In total 132 MRSA positive samples were further investigated by *spa* typing and determination of antimicrobial resistance. The high antimicrobial resistance was evident against penicillin, tetracycline, erythromycin and gentamicin, but lower – against amoxicillin combined with clavulanic acid trimethoprim/sulphamethoxazole and ciprofloxacin. Comparing farms to slaughterhouses, the higher antimicrobial resistance was detected in slaughterhouses (see Figure 3).

Analysing most frequently isolated MRSA *spa* types we found out, that *sea* distribution varied from 42.9% (*spa* type t11744) to 100.0% (*spa* type t899 and t127) while *sec* distribution varied from 8.3% (*spa* type t337) to 14.3 (*spa* type t11744) and *seh* distribution from 24.9% (*spa* type t337) to 100% (*spa* type t2451) (see Table 4 and Figure 4, 5, 6 and 7). Despite of the fact that all of MRSA *spa* type t899 isolates had *sea*, this *spa* type had lowest antimicrobial resistance - resistant only to penicillin, tetracycline and oxacillin, while other MRSA *spa* types were resistant to at least 7 different antibiotics. MRSA *spa* types t1744 and t1333, which show the highest antimicrobial resistance and in the same time are able to produce SEA, SEC and SEH, but *spa* type t127, that has lower antimicrobial resistance not only is able to produce SEA, SEC and SEH, but also can produce SEB.



Pen–penicillin, AmCl–amoxicillin with clavulanic acid, Cef–cephalexin, Cip–ciprofloxacin, Clin–clindamycin, Ery–erythromycin, Ge–gentamicin, Me–meropenem, Tetr–tetracycline, Tri–Trimethoprim/sulphamethoxazole, Van–vancomycin.

Fig.3. **Antimicrobial resistance of MRSA strains isolated from farms and slaughterhouses**

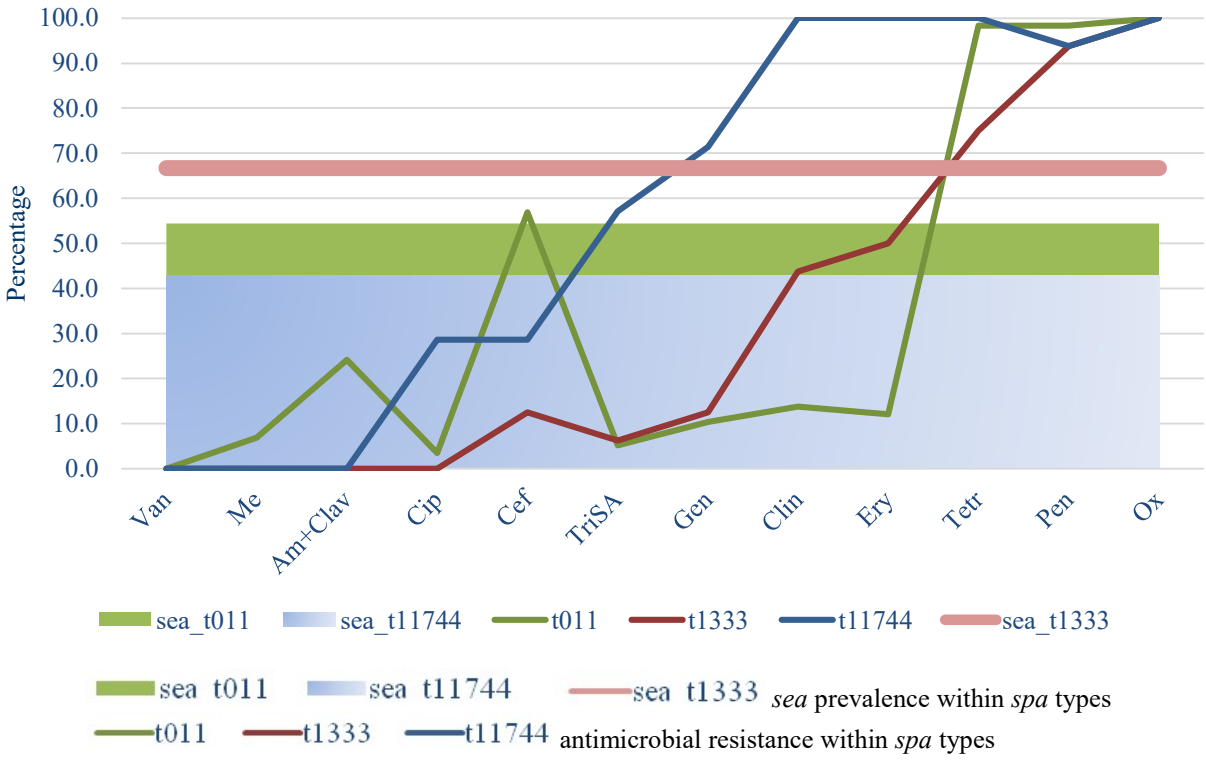


Fig. 4. **Comparison of *sea* and antimicrobial resistance in MRSA *spa* types t11744, t1333 and t011**

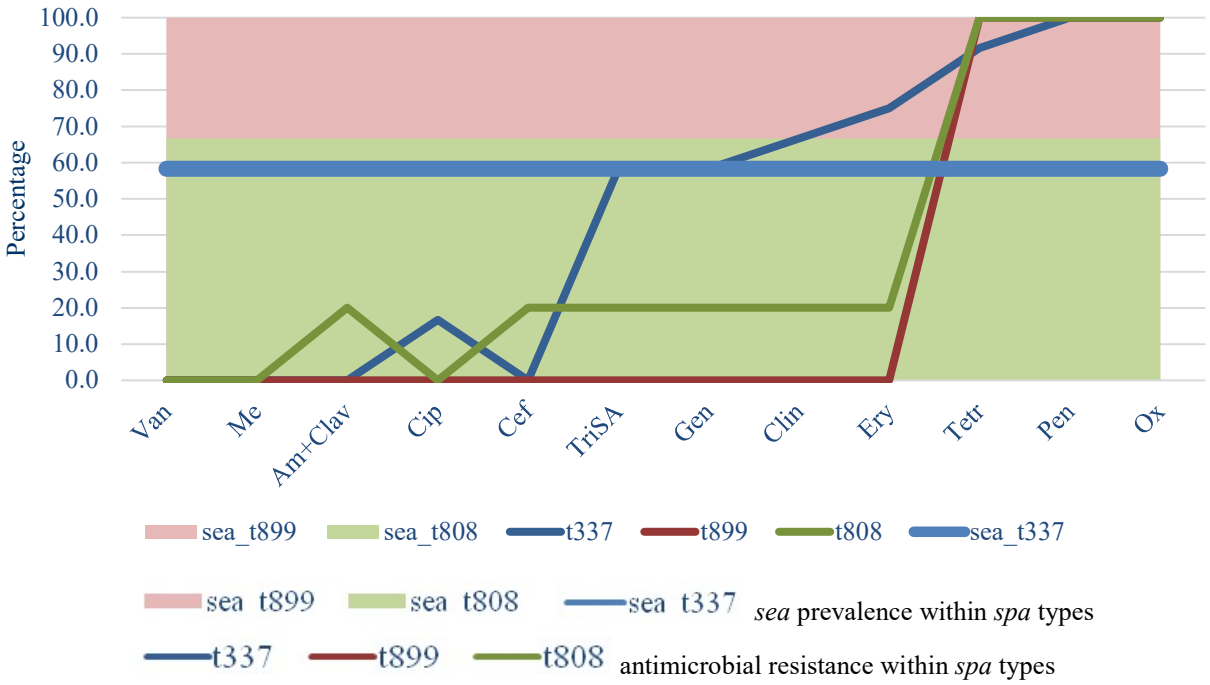


Fig. 5. **Comparison of *sea* gene and antimicrobial resistance in MRSA *spa* types t808, t337 and t899**

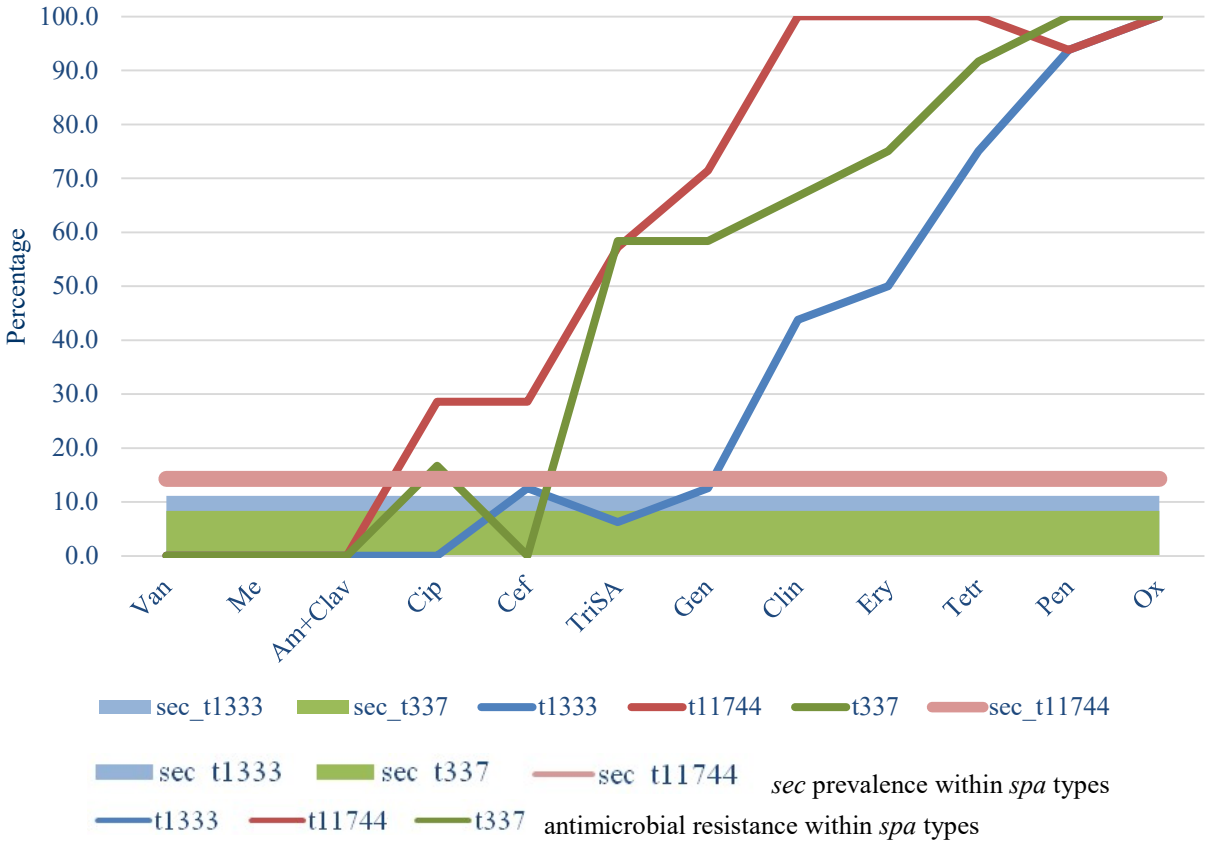


Fig. 6. **Comparison of *sec* gene and antimicrobial resistance in MRSA *spa* types t1333, t337 and t11744**

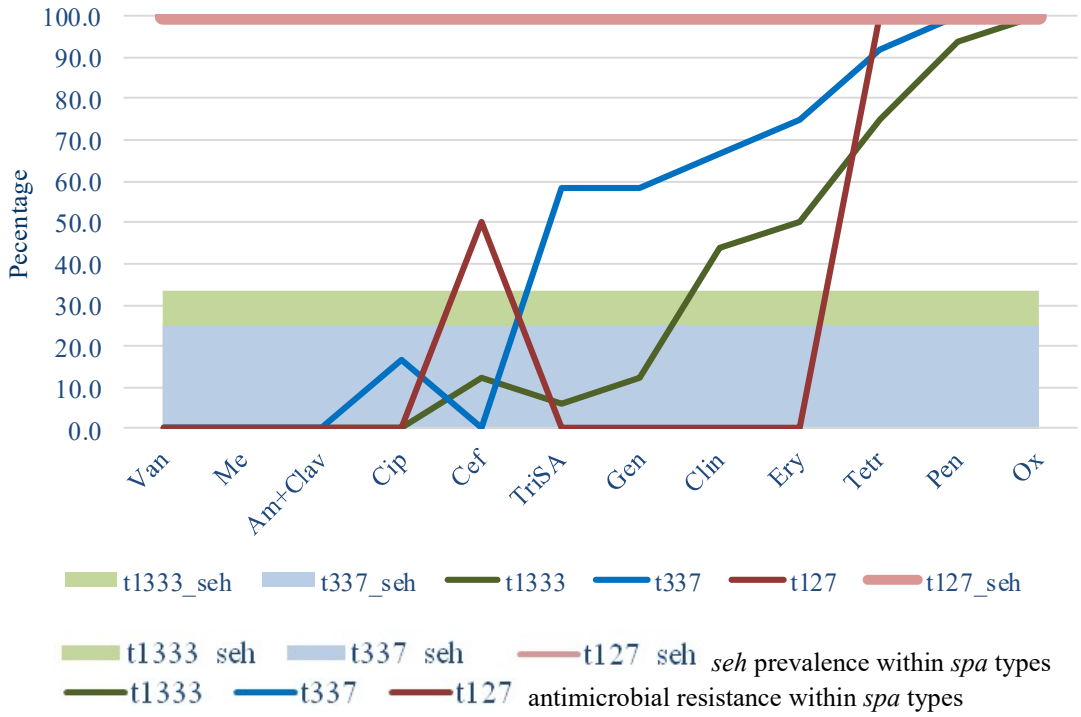


Fig. 7. **Comparison of *seh* gene and antimicrobial resistance in MRSA *spa*types t1333, t337 and t127**

Discussion. Many different foods can be a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning. In any case the main sources of contamination are humans (handlers contaminate food via manual contact or via the respiratory tract by coughing and sneezing) and contamination occurs after heat treatment of the food. Nevertheless, in food such as raw meat, contamination from animal origins are more frequent and due to animal carriage or to infections (le Loir et al. 2003), and as it is seen from our study – the working close to animals make a risk for both – human and animal, and exchange between different MRSA *spa* types my occur via close contact, equipment or dust. Moreover, the slaughterhouses is the place, where animals from different farms, even countries are gathered for slaughter and the widest spectrum of MRSA isolates with different genome types are seen as well as the great chance of MRSA genome exchanges combining different *spa* types in workers as carriers my occur. The additional risk point in slaughterhouses is possibility to contaminate carcasses with both – human and livestock associated MRSA types. Good working practise in slaughterhouses allows to avoid from contamination and decrease MRSA further distribution on carcasses. As it is seen from our study 51.0% of pigs were MRSA carriers while MRSA evidence in samples from carcasses was only 6.67%, but the much lower prevalence is seen in Lithuania (Ružauskas et al. 2014), where only 4 pig origin isolates from 520 (0.8%) tested samples were MRSA positive. In pork *S. aureus* and MRSA rates vary greatly (Agerso et al. 2011), and our study agrees with reports where *S. aureus* is find in 5.0% of the all investigated samples (Agerso et al. 2011), while in other studies more than half of fresh pig meat samples have also been reported to be positive *S. aureus* (Atanassova et al. 2001).

Similar to China research (Chao et al. 2015), where 54.4% of all *S. aureus* isolates harboured SE genes, in our study 52.3% of all MRSA isolates were carrying SE genes, moreover as the tested ones were only MRSA the evidence of SE genes might be higher in *S. aureus* isolates.

Opposite to statement (Pinchuk et al. 2010), that *sec* is commonly isolated from animals, but similar to China research (Chao et al. 2015), where s*ec* was not found in animals, we detected *sec* in 5.9% of all MRSA samples and only 7.2% MRSA positive pigs carried MRSA strain that was capable to produce *sec,* while s*ea*, that is mentioned in statement above as a most common toxin associated with staphylococcal food poisoning coding gene was definitely more spread- respectively in 48.4% from all MRSA isolates and between 50.4% in all MRSA positive pigs. Our study also disagree with other studies (Smyth et al. 2005, Monecke et al. 2007, Ikawaty et al. 2010), where MRSA *sea* gene between isolates from cows were not found, but in high rates were seen *sec*- 19 from 19 cows, 3 from 15 and 19 from 25. Other researchers (Hallin et al. 2011, Huber et al. 2011) found no isolates containing *sea* or *sec* gene in cows and pigs, as well as in China researchers study (Chao et al. 2015) 10.7% and in Huber study (Huber at al. 2010) 5.9% of all MRSA isolates from pigs carried *sea* gene, but no one carried *sec*. In other studies *seh* gene prevalence varried – 5.4% in broiler chicken (Wendlandt et al. 2013), but USA data (Lubna et al. 2015) show high prevalence in pigs – 31.3%.

Opposite to China research (Chao et al. 2015), where *sec* gene were not detected in pigs and other animals, but *seh* gene only in chickens and ducks (1.13% in both species), we found *sec* and *seh* genes in our MRSA pig isolates in 7.2% and 10.8% of animals.

The high rates of *sea* in MRSA isolates from hospital patients were detected in Germany, while in samples from pigs only in 3.0% and in samples from occupationally exposed – 6.3% (2 from 32) (Mutters et al. 2016), but in our study in 5 from 7 MRSA positive isolates from workers. Data from other countries show higher distribution of *sea* genes among MRSA isolates -33.0% in China (Wang et al. 2013), 17.5% in Malaysia (Kim et al. 2006), 27.0% in Korea (Peck et al. 2009), 12.0% in Czech (Sila et al. 2009) and 30.0% in Turkey (Demir et al. 2011).

Despite of other researcher findings (Holtfreter et al. 2007, Varshney et al. 2009, Chao et al. 2015) where mostly appears carriage of multiple SE genes approximately in 80% of isolates, in our study only 22.22% of all isolates that contained SE genes carried two or more SE genes.

China researchers (Chao et al. 2015) found that even between on MRSA *spa* type virulence genes varied remarkably and MRSA *spa* type t127 had *sea, seb* and *seh* genes as well as in our study such a situation was seen, but in addition we detected also the evidence of *sec* gene in this *spa* type. Opposite to China study (Chao et al. 2015) we found that MRSA *spa* types t011 and t899 carry *sec* gene.

In our study *sea* in MRSA isolates appears most frequently and agrees with other studies and reports, where *sea* is mainly isolated from all types of products, including meat, milk products and even contaminated vegetables: 359 outbreaks that occurred in United Kingdom between 1969 and 1990 revealed 79% of the *S. aureus* strains produced SEA (Wieneke et al. 1993). SEA was also the enterotoxin most frequently found among 31 staphylococcal food poisoning outbreaks in France (69.7) in great variety of foods between 1981 and 2002 (Kerouanton 2007) and in other outbreaks, in Austria, USA, Brazil (Veras et al. 2008), in outbreaks in Taiwan during 2001-2003, in Korea 90% of food isolates (Chiang et al. 2008).

Conclusion. The higher prevalence of MRSA was found in slaughterhouses as in farms, and from our point of view the reason for this is high contact possibility between animals taken from several different farms and regions to slaughter as well as developing high colonization risk for stuff too. Contrary to other researcher studies, we detected high *sea* gene distribution in MRSA isolates from pigs and only some isolates with *sec* gene, moreover among MRSA *spa* types t1333 and t899 with highest *sea* gene distribution, antimicrobial resistance against same antibiotics (thrimetho-prim/sulphamethozole, gentamicin, clindamycin and erythromycin) was lower. MRSA *spa* types t1333 and t011, which had highest prevalence, in the same time, comparing to other MRSA *spa* types, had lower antimicrobial resistance level within *spa* type, but MRSA *spa* type t337, that was third most spread MRSA *spa* type, in the same time showed high antimicrobial resistance (resistance to 9 different antibiotics) and the resistance level within *spa* type was higher. Mostly all MRSA *spa* types showed high resistance level to penicillin and tetracycline that were the first choice of antimicrobials in Latvian pig farms from long ago till now.

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