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Comparative Analysis of Superantigen Genes in *Staphylococcus xylosus* and *Staphylococcus aureus* Isolates Collected from a Single Mammary Quarter of Cows with Mastitis

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The purpose of this study was to analyze and compare genes encoding superantigens (SAgs) in *Staphylococcus xylosus* and *Staphylococcus aureus* isolates collected simultaneously from milk of the same cows with clinical mastitis. Genes encoding staphylococcal enterotoxins and enterotoxin-like proteins (*sea-selu*), toxic shock syndrome toxin 1 (*tst-1*) and exfoliative toxins (*eta* and *etd*) were investigated. It was found that among 30 isolates of *S. xylosus*, 16 (53.3%) harbored from 1 to 10 SAg genes. In total, in 16 SAg positive *S. xylosus*, 11 different enterotoxin genes were detected: *sec*, *sed*, *seg*, *seh*, *sei*, *selm*, *seln*, *selo*, *selp*, *ser*, *selu* and one *etd* gene encoding exfoliative toxin D. The most prevalent genes were *ser*, *selu*, and *selo*. Among all the positive isolates of *S. xylosus*, a total of 14 different SAg gene combinations were detected. One combination was repeated in 3 isolates, whereas the rest were detected only once. However, in the case of *S. aureus* all the 30 isolates harbored the same combination of SAg genes: *seg*, *sei*, *selm*, *seln*, *selo* and on the basis of PFGE analysis all belonged to the same clonal type. Also noteworthy was the observation that SAg genes detected in *S. aureus* have also been found in *S. xylosus*. The findings of this study further extend previous observations that SAg genes are present not only in *S. aureus* but also in coagulase-negative staphylococci, including *S. xylosus*. Therefore, taking into account that the SAg genes are encoded on mobile genetic elements it is possible that these genes can be transferred between different species of coexisting staphylococci.

***Keywords*:** superantigen genes, enterotoxins, *S. xylosus*, *S. aureus*, mastitis

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Introduction

One of the most common mastitis-causing pathogens worldwide is *Staphylococcus aureus* (Younis *et al.*, 2005). The pathogenic capacity of a particular *S. aureus* strain is attributed to a combination of invasive properties and extracellular factors such as toxin production. *S. aureus* produces a wide variety of toxins including toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins (ETA to ETD), staphylococcal enterotoxins (SEs; SEA to SEE, SEG to SEI and SER) with demonstrated emetic activity, and staphylococcal enterotoxin-like (SEl) proteins, which are not emetic in a primate model (SElL and SElQ) or have yet to be tested (SElJ, SElK, SElM to SElP, SElU and SElV). At present it is assumed that there are 23 serologically different SEs and SEls (Vasconcelos *et al.*, 2011). All the toxins listed above possess superantigenic activity and were designated as staphylococcal superantigens (SAgs). The SAg genes are encoded by accessory genetic elements, including plasmids, phages, *S. aureus* pathogenicity island (SaPI), *v*Sa genomic islands, or by genes located next to the staphylococcal cassette chromosome (SCC) implicated in methicillin resistance (Vasconcelos and da Cunha, 2010; Wu *et al.*, 2011).

Coagulase-negative staphylococci (CoNS) are a diverse group of commensals inhabiting the skin and mucous membranes of humans and animals. However some species of CoNS are known as important opportunistic human pathogens. The role of CoNS as animal pathogens is less understood. However, some of the CoNS species are involved in diseases of various animals, including mastitis in cows (da Cunha *et al.*, 2007; Park *et al.*, 2011). According to some authors, *Staphylococcus xylosus* is one of the most prevalent CoNS species involved in bovine intramammary infections (IMI) (Malinowski *et al.*, 2006; Feßler *et al.*, 2010) and one with the highest percentage of genes encoding enterotoxins (Park *et al.*, 2011).

SEs, SEls and TSST-1 are exotoxins originally identified in *S. aureus*, but they are also detected in CoNS, including strains isolated from the mammary gland of cattle and other ruminants (Park *et al.*, 2011). Several authors have suggested the presence of enterotoxin and enterotoxin-like genes in genomes of *S. xylosus*, *S. chromogenes*, *S. saprophyticus*, *S. lentus*, *S. warneri*, *S. sciuri* and *S. haemolyticus*, *S. hyicus*, *S. simulans*, *S. epidermidis*, *S. succinus*, *S. capitis,* whereas the *tst-1* gene was detected in *S. xylosus*, *S. saprophyticus*, *S. warneri*, *S. hominis*, and *S. haemolyticus* (Nedelkov and Nelson, 2003; Park *et al.*, 2011).

According to Park *et al.* (2011) CoNS isolates from bovine

IMI may be a possible reservoir of superantigenic toxin genes typically identified in *S. aureus*. It is well documented, that SAg genes that are located on the mobile genetic elements can spread among *S. aureus* isolates. It is also considered that these genes may be exchanged between *S. aureus* and *other staphylococci* (Ławrynowicz-Paciorek *et al.*, 2007; Vasconcelos and da Cunha, 2010).

Therefore, to add further support to these hypotheses and to extend previous observations, in the present study we attempted to analyze the genes encoding SAgs in *S. aureus* and *S. xylosus* isolated simultaneously from milk of cows with clinical mastitis.

Materials and Methods

Bacterial isolation and species identification

Prior to this study, 30 *Staphylococcus aureus* and 30 *Staphylococcus xylosus* isolates were collected from the milk of 30 different cows with clinical mastitis in one herd from Western Pomerania (Poland). Both *S. aureus* and *S. xylosus* isolates were collected from a single mammary quarter at a single point in time. The isolates of *S. aureus* and *S. xylosus* that were collected independently from different samples of milk were not considered.

Table 1. Primers and expected size of PCR products (bp) of investigated genes

	Gene	Primers	Sequence (5′→3′)	PCR product (bp)	References
Multiplex I	*sea*	sea-1	gaa aaa agt ctg aat tgc agg gaa ca	560	Jarraud *et al.* (2002)
		sea-2	caa ata aat cgt aat taa ccg aag gtt c		
	seh	seh-1	caa tca cat cat atg cga aag cag	376	Jarraud *et al.* (2002)
		she-2	cat cta ccc aaa cat tag cac c		
	sec	sec-1	ctt gta tgt atg gag gaa taa caa aac atg	275	Jarraud *et al.* (2002)
		sec-2	cat atc ata cca aaa agt att gcc gt		
	tst-1	tst-1	ttc act att tgt aaa agt gtc aga ccc act	180	Jarraud *et al.* (2002)
		tst-2	tac taa tga att ttt tta tcg taa gcc ctt		
Multiplex II	*sed*	sed-1	gaa tta agt agt acc gcg cta aat aat atg	492	Jarraud *et al.* (2002)
		sed-2	gct gta ttt ttc ctc cga gag t		
	etd	etd-1	caa act atc atg tat caa gga tgg	358	Zhang *et al.* (1998)
		etd-2	cca gaa ttt ccc gac tca g		
	eta	eta-1	act gta gga gct agt gca ttt gt	190	Jarraud *et al.* (2002)
		eta-2	tgg ata ctt ttg tct atc ttt ttc atc aac		
	selk	sek-1	atg cca gcg ctc aag gc	134	Holtfreter *et al.* (2007)
		sek-2	aga ttc att tga aaa ttg tag ttg att agc t		
		sek-3	tgc cag cgc tca agg tg		
Multiplex III	*see*	see-1	caa aga aat gct tta agc aat ctt agg c	482	Jarraud *et al.* (2002)
		see-2	cac ctt acc gcc aaa gct g		
	seb	seb-1	att cta tta agg aca cta agt tag gga	404	Jarraud *et al.* (2002)
		seb-2	atc ccg ttt cat aag gcg agt		
	selm	sem-1	cta tta atc ttt ggg tta atg gag aac	326	Jarraud *et al.* (2002)
		sem-2	ttc agt ttc gac agt ttt gtt gtc at		
	sell	sel-1	gcg atg tag gtc cag gaa ac	234	Holtfreter *et al.* (2007)
		sel-2	cat ata tag tac gag agt tag aac cat a		
	selo	seo-1	agt ttg tgt aag aag tca agt gta ga	180	Jarraud *et al.* (2002)
		seo-2	atc ttt aaa ttc agc aga tat tcc atc taa c		
Multiplex IV	*seln*	sen-1	cgt ggc aat tag acg agt c	474	Holtfreter *et al.* (2007)
		sen-2	gat tga tyt tga tga tta tka g		
	seg	seg-1	tct cca cct gtt gaa gg	323	Holtfreter *et al.* (2007)
		seg-2	aag tga ttg tct att gtc g		
	selq	seq-1	acc tga aaa gct tca agg a	204	Holtfreter *et al.* (2007)
		seq-2	cgc caa cgt aat tcc ac		
	selj	sej-1	tca gaa ctg ttg ttc cgc tag	138	Holtfreter *et al.* (2007)
		sej-2	gaa ttt tac cay caa agg tac		
Multiplex V	*sei*	sei-1	cty gaa ttt tca acm ggt ac	461	Holtfreter *et al.* (2007)
		sei-2	agg cag tcc atc tcc tg		
	ser	ser-1	agc ggt aat agc aga aaa tg	363	Holtfreter *et al.* (2007)
		ser-2	tct tgt acc gta acc gtt tt		
	selu	seu-1	aat ggc tct aaa att gat gg	215	Holtfreter *et al.* (2007)
		seu-2	att tga ttt cca tca tgc tc		
	selp	sep-1	gaa ttg cag gga act gct	182	Holtfreter *et al.* (2007)
		sep-2	ggc ggt gtc ttt tga ac		

Table 2. ***S. aureus*** **control strains used in this study**

Strains	Superantigen gene(s)	References
FRI1151m	*sed*, *selj*, *ser*	Holtfreter *et al.* (2007)
FRI913	*sea*, *sec*, *see*, *selk*, *sell*, *selq*, *tst-1*	Wu *et al.* (2011)
N315	*sec*, *seg*, *sei*, *sell*, *selm*, *seln*, *selo*, *selp*, *tst-1*	Kuroda *et al.* (2001)
FRI137	*sec*, *seh*, *sell*, *selu*	Wu *et al.* (2011)
TY114	*Etd*	Wu *et al.* (2011)
A920210	*Eta*	Wu *et al.* (2011)
Col	*seb*, *selk*, *selq*	Wu *et al.* (2011)
8325-4	no SAg genes	Holtfreter *et al.* (2007)

The dairy farm consisted of about 400 Polish Holstein-Friesian cows. The samples of milk were collected over a period of one year. The diagnoses of clinical mastitis were provided by the local veterinarian. Mastitis was classified as clinical if any systemic symptoms or local signs or alterations in milk appearance were detected.

The isolates were identified as *S. aureus* or *S. xylosus* on the basis of their biochemical properties (api STAPH, bioMérieux, France), their ability to coagulate rabbit plasma and to produce a clumping factor (Biomed, Poland). All of the isolates were also examined by PCR using primers for the *S. aureus* specific *coa* gene, as described by Aarestrup *et al.* (1995) and for the *S. xylosus* specific *gehM* gene according to Iacumin *et al.* (2007). All bacterial strains were stored in 10% glycerol solution in Tryptone Soya Broth (TSB, Oxoid, UK) at -20°C.

DNA extraction

All bacterial isolates were plated onto Columbia agar base with 5% sheep blood (Grasso, Poland) and cultivated for 24 h at 37°C. After incubation, one colony-forming unit of each isolate was transferred into Luria-Bertani broth (Oxoid) and incubated for 24 h at 37°C. After cultivation, the optical density (at 600 nm) of bacterial cultures was adjusted to 1.0. Finally, total DNA was extracted from bacterial cultures using the Genomic Mini kit (A&A Biotechnology, Poland).

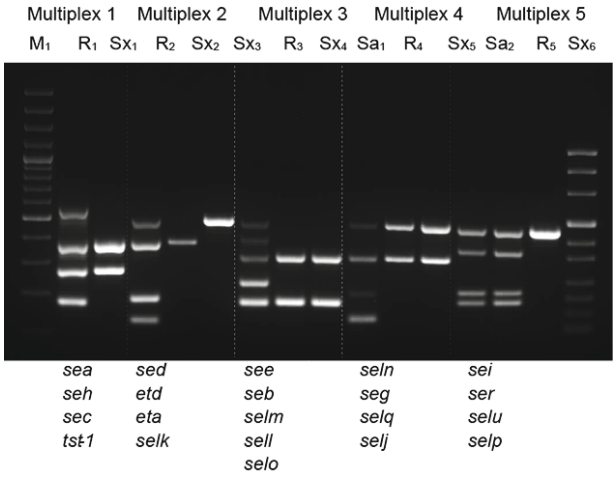

Fig. 1. Detection of staphylococcal superantigen genes from *S. xylosus* and *S. aureus* isolates by multiplex PCR. M_1, Molecular mass marker (MassRuler Express DNA Ladder, 10000-100 pz, Fermentas, Lithuania); M_2, Molecular mass marker (pUC Mix Marker 8, 1118-67 pz, Fermentas); R_1-R_5, positive controls for each multiplex PCR; Sx_1-Sx_6, representative amplicons for *S. xylosus*: Sx_1, *seh*, *sec*; Sx_2, *etd*; Sx_3, *sed*; Sx_4, *selm*, *selo*; Sx_5, *seln*, *seg*; Sx_6, *sei*, *ser*, *selu*, *selp*; Sa_1-Sa_3: representative amplicons for *S. aureus*: Sa_1, *selm*, *selo*; Sa_2, *seln*, *seg*; Sa_3, *sei*.

Multiplex PCR

The presence of genes encoding enterotoxins, enterotoxin-like proteins, exfoliative toxins and TSST-1 in the investigated *S. aureus* and *S. xylosus* was determined by multiplex PCR as described by Zhang *et al.* (1998), Jarraud *et al.* (2002), and Holtfreter *et al.* (2007). The combinations of

Table 3. Primers and expected size of PCR products (bp) of investigated genes

Species (n)	Gene combinations	*etd*	*sec*	*sed*	*seg*	*seh*	*sei*	*selm*	*seln*	*selo*	*selp*	*ser*	*selu*	No. of isolates
S. xylosus (30)	Negative for toxin genes													14
	ser											+		1
	seg, *seln*				+				+					1
	selm, *selo*							+		+				1
	ser, *selu*											+	+	3
	seg, *sei*, *selo*				+		+			+				1
	sei, *ser*, *selu*						+					+	+	1
	sei, *selm*, *selo*						+	+		+				1
	sec, *seh*, *ser*, *selu*		+			+						+	+	1
	sed, *seg*, *selo*, *ser*			+	+					+		+		1
	sei, *selp*, *ser*, *selu*						+				+	+	+	1
	selm, *selo*, *ser*, *selu*							+		+		+	+	1
	sei, *selm*, *selo*, *ser*, *selu*						+	+		+		+	+	1
	etd, *sec*, *seh*, *sei*, *selm*, *selo*, *ser*, *selu*	+	+			+	+	+		+		+	+	1
	etd, *sec*, *seg*, *seh*, *sei*, *selm*, *seln*, *selo*, *ser*, *selu*	+	+		+	+	+	+	+	+		+	+	1
	Gene occurrence	2	3	1	4	3	7	6	2	8	1	12	10	16
S. aureus (30)	Negative for toxin genes													0
	seg, *sei*, *selm*, *seln*, *selo*				+		+	+	+	+				30
	Gene occurrence				30		30	30	30	30				30

primers and the expected size of the multiplex PCR products for each of the investigated genes are presented in Table 1.

Each reaction mixture (25 µl), for both *S. aureus* and *S. xylosus*, consisted of 1 U AmpliTaq Gold DNA Polymerase with 1X PCR buffer (Applied Biosystems Inc., USA), 100 nM of dNTP mix (Applied Biosystems Inc.), 0.15 to 0.4 µM of each primer and 20–50 ng of template DNA. The multiplex PCR for SAg genes was carried out with the following thermal cycling conditions: an initial denaturation of DNA at 95°C for 10 min was followed by 35 cycles of amplification (95°C for 30 sec, 55°C for 45 sec, and 72°C for 60 sec), ending with a final extension at 72°C for 10 min. All PCR products were characterized by 1.5% agarose gel electrophoresis in 1X Tris-borate-EDTA buffer, followed by staining with ethidium bromide, visualization under UV light and analysis using GeneTools software (Syngene, UK).

The mixture of genomic DNA from seven *S. aureus* reference strains was used for multiplex PCR of each set as a positive control. As the negative control, genomic DNA from *S. aureus* without SAg genes was used (Table 2). Amplification of non-template controls was also included in each analysis to determine if DNA contamination had occurred.

Analysis of the molecular diversity among *S. aureus* isolates

Analysis of the genetic relationships among the examined isolates was performed by digestion of chromosomal DNA with *Sma*I enzyme and separation of the DNA by pulsed field gel electrophoresis (PFGE), according to the Centers for Disease Control and Prevention protocol (CDC, www.cdc.gov website). In the first step, single colonies of *S. aureus* isolates were transferred from Columbia agar base with 5% sheep blood (Grasso) to 2 ml of TSB and incubated for 24 h at 37°C. Next, 90 µl of each culture were transferred to 1.5 ml tubes and centrifuged at 10,000 rpm for 2 min, then the resulting cell pellets were mixed with 10 µl of lysozyme (Sigma-Aldrich, Germany), 4 ml of lysostaphin (DNA-Gdańsk, Poland) and 300 µl of 2% liquid agarose solution warmed to 55°C (Bio-Rad Laboratories, France) and transferred into molds to form blocks, which were further treated with proteinase K (DNA-Gdańsk), and digested with the restriction enzyme *Sma*I (Thermo Scientific, Germany). These steps were carried out separately in two replications for each of the test strains of *S. aureus*. The blocks were loaded onto 1% agarose gels and electrophoresed in TBE buffer (Inno-Train Diagnostik GmbH, Germany) using a CHEF-DR apparatus (Bio-Rad Laboratories). Run time was 20 h with an initial switch time of 5 sec and a final switch time of 40 sec. The ramping factor was linear. Temperature was set at 14°C, voltage at 6 V/cm, and the included angle at 120°. Ethidium bromide was used as the DNA stain. Bands were visualized under UV light and a photograph was taken using the Molecular Imager ChemiDoc XRS (Bio-Rad Laboratories). The image was

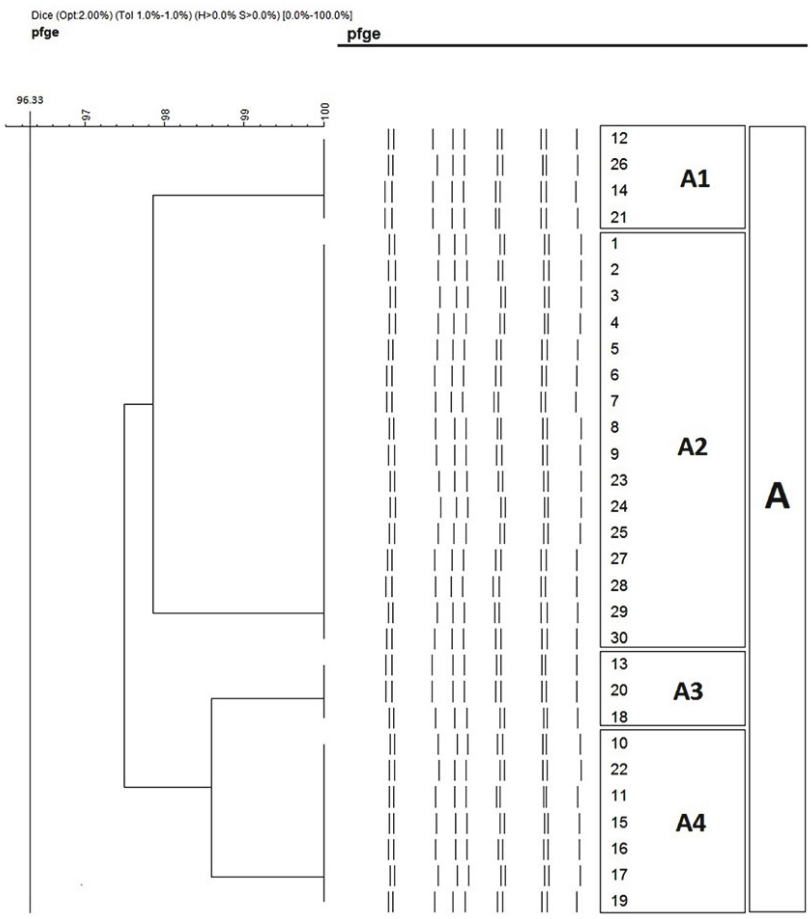

Fig. 2. Dendrogram of PFGE clusters and genotypic relationships of *S. aureus* isolates. Cut-off point is equal to 96.33 (Sab = 96.33%).

analyzed by FPQuest Software, version 4.5 (Bio-Rad Laboratories). The dendrogram was constructed using the Dice correlation coefficient, the unweighted pair group method and arithmetic mean with a 2% band tolerance.

Results

Multiplex PCR

The multiplex PCR used in this study enabled amplification of all investigated SAg genes including enterotoxins, staphylococcal-like proteins and also genes for TSST-1 and two variants of exfoliative toxins in DNA from control *S. aureus* strains. The PCR products from the positive control were equal to those previously described and agreed with their expected size. For no-template and negative controls, no amplicon was generated and nonspecific reactions were not observed. All multiplex sets used in this study allowed amplification of SAg genes from the investigated *S. aureus* and *S. xylosus* isolates without any nonspecific amplifications (Fig. 1).

Prevalence and distribution of SAg genes

Among 30 *S. xylosus* isolates, 16 (53.3%) showed the presence of at least one of the SAg genes. In total, in all *S. xylosus* isolates, 12 different SAg genes were amplified. The most frequently identified genes were: *ser* gene, confirmed in 12 isolates of the 16 positive strains (12/16, 75.0%), *selu* gene found in 10 isolates (10/16, 62.5%) and *selo* gene identified in 6 isolates (6/16, 37.5%). In addition, the following genes were identified: *sec* (3/16, 18,8%), *etd* (2/16, 12.5%), *sed* (1/16, 6.3%), *seg* (3/16, 18.8%), *seh* (3/16, 18.8%), *sei* (5/16, 31.3%), *selm* (5/16, 31.3%), *seln* (2/16, 12.5%), and *selp* (1/16, 6.3%). A total of 14 different SAg gene combinations were observed among the 16 SAg positive *S. xylosus* isolates. The most common SAg gene combination was *ser* and *selu*, which was present in 3 isolates (3/16, 18.8%) (Table 3). Four isolates (4/16, 25.0%) had both classical and newly described SAg genes. Twelve isolates (12/16, 75.0%) had only newly described SAg genes, whereas isolates with only classical SAg genes were not found. In contrast, in all of the 30 *S. aureus* isolates, the same set of SAg genes: *seg*, *sei*, *selm*, *seln* and *selo* was detected.

Genotypic relationships of *S. aureus* isolates

In order to confirm whether *S. aureus* isolates that harbor the same set of superantigen genes belong to a single clonal type, macrorestriction analysis of DNA using pulsed field gel electrophoresis was performed. It showed that all isolates belonged to one PFGE type, identified by an upper case A, and thus according to established criteria, constituted one clone with a genetic similarity coefficient (Sab) of 96.33% (Fig. 2). For classification of the isolates, those with identical patterns were considered representatives of a single PFGE type. Additionally, within type A, four PFGE subtypes, marked with numbers next to the letter A (A1-A4) were also distinguished.

Discussion

Staphylococci can produce a large group of toxins called superantigens (SAgs). Superantigens are microbial virulence factors that have strong effects on the immune system through the subversion of immune responses and delays in the establishment of pathogen-specific immunity. Although staphylococcal SAgs are typically associated with *S. aureus*, it was found that these microbial agents can be produced by many CoNS species (da Cunha *et al.*, 2007). However, little information has been reported on the prevalence and genetic constructs for SAg genes in *S. xylosus*. To extend previous observations and to build upon the previous investigations, in this study we screened for the presence of all currently known staphylococcal SAg genes in *S. aureus* and *S. xylosus*. We considered a special case, when both staphylococcal species coexisted in the mammary glands. *S. xylosus* was chosen for this research as it was found to be the most prevalent CoNS in the investigated herd. In this study, *S. aureus* and *S. xylosus* were simultaneously isolated from the quarter milk in 70% of all the milk samples tested and were also the most frequent bacterial species simultaneously identified in a sample of milk (data not shown).

Current studies also revealed that all the collected isolates of *S. aureus* harbored the same combination of SAg genes: *seg*, *sei*, *selm*, *seln*, *selo* and on the basis of PFGE analysis they all belonged to clonal type A. A slight differentiation of isolates belonging to the A type resulted in the splitting of this type into 4 subtypes. This division may indicate a gradual evolution of the epidemic strain as has been previously suggested by McDougal *et al.* (2003). Consistent with previous studies (Haveri *et al.*, 2008; Nawrotek *et al.*, 2012), we showed that one clonal type of bacterium, carrying the same set of genes encoding particular virulence factors, can be dominant among *S. aureus* isolates collected from one herd in a relatively short time. The spreading in the environment of only certain *S. aureus* genotypes, as previously suggested and described by Annemüller *et al.* (1999) and Zschöck *et al.* (2005), is achieved by clones having specific virulence and resistance against host defense mechanisms.

Our findings also showed that one or more classical and/or newly described SAg genes were widely distributed in 16 (53.3%) *S. xylosus* isolates. In this case, 14 different SAg gene combinations were observed. However, only one combination of SAg genes was repeated, in 3 isolates, whereas the others were found only in individual strains. This is consistent with the study of Nawrotek *et al.* (2010) who identified 51.4% of *S. xylosus* among 35 CoNS isolates collected from milk of cows with clinical mastitis on a farm in the north-west of Poland. According to these authors, 38.9% of analyzed *S. xylosus* isolates harbored SAg genes, among which *sea* (71.4%), *sec* (14.3%), and *sed* (14.3%) were identified most frequently. In addition, in 71.4% of the positive *S. xylosus*, these authors obtained PCR amplicons, which on the basis of the *in silico* analysis, were identified as: *seg*, *seh*, *sei*, or *selu*. Similarly, Park *et al.* (2011) reported the presence of one or more SAg genes in 31.2% of all CoNS isolated from cows with mastitis. In total, these authors detected 21 different combinations of SAg genes. The most common SAg gene combination was: *seb*, *seln*, and *selq*, found in

54.9% of SAg-positive isolates, including *S. xylosus*. In addition, these authors did not detect genes for *see*, *sell*, *selm*, *selp*, and *tst-1*. Furthermore, according to Park *et al.* (2011), SAg genes were found in 9 of the 11 identified species of CoNS. *S. xylosus* was the second (after *S. chromogenes*) most frequently isolated CoNS species and it was the species with the highest percentage of SAg genes (45.8%). The SAg genes found by these authors in *S. xylosus* were *seb*, *seln*, *selq*, *seg*, *selo*, and *selu* and the most prevalent gene combination was *seb*, *seln* and *selq*, which was found in 8 (72.7%) isolates.

It is widely agreed that SAg genes are encoded by accessory genetic elements, including plasmids, phages, staphylococcal pathogenicity islands, *v*Sa genomic islands or by genes located next to the staphylococcal cassette chromosome (SCC) (LeLoir *et al.*, 2003; Ławrynowicz-Paciorek *et al.*, 2007). The association of SAg genes with mobile genetic elements facilitates their horizontal transfer among different strains of staphylococci, and constitutes an important role in the evolution of *S. aureus* and CoNS as pathogens (Ławrynowicz-Paciorek *et al.*, 2007; Vasconcelos and da Cunha, 2010). It can be noted that all *S. aureus* SAg genes (*seg*, *sei*, *selm*, *seln*, *selo*, *selu*) detected in our study are located within *egc* (enterotoxin gene cluster) 1 or *egc* 2 *v*Sa genomic islands or, with the exception of the *selm* gene, they can be located on *egc* 3 (*seg*, *sei*, *seln*, *selo* and *selu*) or *egc* 4 (*seg*, *seln* and *selo*). The gene set consisting of *seg*, *sei*, *selm*, *seln*, *selo* and *selu* was also found in one isolate of *S. xylosus*, whereas in the remaining isolates these genes were present in different numbers and combinations.

The present study also revealed that 12 isolates of *S. xylosus* harbored the plasmid *ser* gene and 1 isolate additionally possessed the plasmid *sed* gene. However, despite the fact that the plasmid genes can be easily transferred among staphylococci of the same and different species, in our study these genes have not been identified in any of the *S. aureus* isolates. The remaining genes: *sec* gene located on *S. aureus* pathogenicity island (SaPI) and phage *sep* gene were detected respectively in 3 and 1 isolate of *S. xylosus*. Both locations are widely distributed in *S. aureus* and have also been found in other species of *Staphylococcus* (Argudín *et al.*, 2010). It was also found that 3 isolates of *S. xylosus* possessed a *seh* gene, which is located on the antibiotic resistance staphylococcal cassette chromosome *mec* (SCC*mec*) and is also subject to interspecies horizontal gene transfer (Argudín *et al.*, 2010; Wu *et al.*, 2011). Additionally, in 2 isolates of *S. xylosus*, we detected the presence of the *etd* gene. This gene, according to Yamaguchi *et al.* (2002), is located on the mobile *etd* pathogenicity island. To our knowledge, this is the first report on the detection of a gene encoding exfoliative toxin in CoNS.

In summary, all *S. aureus* isolates included in our experiment had the same set of genes encoding SAgs and belonged to a single PFGE clonal type. By contrast, in *S. xylosus* there was a large variety in the SAg genes detected and only one set of SAg genes was repeated, in 3 (18.8%) isolates. Interestingly, the total number of different SAg genes detected in *S. xylosus* (11) was more than two times higher than the number of different SAg genes identified in *S. aureus* (5). In addition, it must be noted that all of the genes found in *S. aureus* were also identified in *S. xylosus*. This may suggest the possibility of horizontal transfer of SAg genes between these staphylococcal lineages. The presence of SAg genes in *S. xylosus* also confirms the possibility of participation of CoNS in the initiation and course of mastitis and suggests a possible role of these bacteria in food intoxication, which is currently particularly identified with *S. aureus*.

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Conflict of interest statement

None of the authors have any conflicts of interest that could inappropriately influence their work.

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