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Virulence gene profiles in *Staphylococcus aureus* isolated from cows with subclinical mastitis in eastern Poland

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Staphylococcus aureus is arguably the most important pathogen involved in bovine mastitis. The aim of this study was to determine the virulence gene profiles of 124 *Staph. aureus* isolates from subclinical mastitis in cows in eastern Poland. The presence of 30 virulence genes encoding adhesins, proteases and superantigenic toxins was investigated by PCR. The 17 different combinations of adhesin genes were identified. Occurrence of *eno* (91·1%) and *fib* (82·3%) genes was found to be common. The frequency of other adhesion genes *fnbA, fnbB, ebps* were 14·5, 50, 25%, respectively, and for *cna* and *bbp* were 1·6%. The *etA* and *etD* genes, encoding exfoliative toxins, were present in genomes of 5·6 and 8·9% isolates, respectively. The *splA* and *sspA*, encoding serine protease, were detected in above 90% isolates. The most frequent enterotoxin genes were *sei* (21%), *sem* (19·4%), *sen* (19·4%), *seg* (18·5%) and *seo* (13·7%). The *tst* gene was harboured by 2·4% isolates. The 19 combinations of the superantigenic toxin genes were obtained and found in 35·5% of isolates. Three of them (*seg, sei, sem, sen, seo; sec, seg, sei, sem, sen, seo* and *seg, sei, sem, sen*) were the most frequent and found in 16·1% of the isolates. The most common virulotype, present in 17·7% of the isolates, was *fib, eno, fnbB, splA, splE, sspA*. The results indicate the variation in the presence of virulence genes in *Staph. aureus* isolates and considerable diversity of isolates that are able to cause mastitis in cows.

Keywords: Bovine mastitis, *Staphylococcus aureus*, virulence genes, virulence profiles.

Mastitis in dairy cattle is the most prevalent disease, and is responsible for losses of milk production and reduction of milk quality.

Staphylococcus aureus is perhaps the most important pathogen involved in bovine mastitis and is common in Polish dairy herds (Bystroń et al. 2009; Kot et al. 2012b; Szweda et al. 2014). Mastitis caused by *Staph. aureus* is the result of production of several virulence factors, that can contribute in different ways of pathogenesis. The virulence factors produced by *Staph. aureus* can be divided into different groups, including degradative enzymes, cell wall-associated adhesins, and superantigenic toxins. The differences in pathogenicity of *Staph. aureus* strains could result from geographical distribution and from host- and tissue-related characteristics (van Leeuwen et al. 2005). The numbers and combination of virulence genes may be important contributions to pathogenic potential of *Staph. aureus* strains (Zecconi et al. 2006). High number of *Staph. aureus* genotypes present in bovine herds worldwide has been studied to develop better strategies of treating mastitis. However, molecular data on *Staph. aureus* causing bovine mastitis in Poland remain scarce. The aim of this study was to examine the presence of 30 important virulence genes encoding adhesins, proteases and superantigenic toxins in 124 of *Staph. aureus* isolates and to determine the genetic profiles specific for *Staph. aureus* causing subclinical mastitis in cows in eastern Poland.

We investigated the presence of seven genes encoding different adhesins to determine if they may be involved in binding of *Staph. aureus* to the epithelial cells of the cows mammary gland. Staphylococcal proteases are important virulence factors, which can cleave host proteins and

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allow the transition of *Staph. aureus* cells from an adhesive to an invasive phenotype, and that's why the presence of the three serine protease-encoding genes was investigated. Although the genes encoding exfoliative toxins are primarily associated with human *Staph. aureus* strains, we also investigated the presence of these genes in our isolates because it is not fully understood whether these toxins are also produced by animal strains.

We also investigated the presence of the genes encoding the superantigenic toxins, which play an important role in modulating the host immune response and would promote the persistence of *Staph. aureus* in bovine mammary gland.

Materials and methods

Bacterial isolates

A total of 124 *Staph. aureus* isolates from subclinical bovine mastitis milk samples were used in this study. The diagnosis of mastitis was made on the basis of clinical examination of the udder by the veterinarian. Additionally, a quarter was identified as infected when somatic cell counts (SCC) increased above 200 000/ml (Malinowski et al. 2006). SCC were measured with Fossomatic™Minor (Foss, Denmark).

All isolates were collected in 2007 and 2008 in the eastern part of Poland. The methods of identification of bacterial isolates as *Staph. aureus* were described previously (Szweda et al. 2012, 2014).

Isolation of DNA

Genomic DNA from the *Staph. aureus* isolates was extracted by using the Genomic Mini DNA kit (A&A Biotechnology-Gdynia, Poland) according to the manufacturer's protocol. 2·5 µl of the total extracted material from each test sample was used as a template DNA for PCR application.

Detection of virulence-associated genes by PCR

Primer sequences for adhesin genes were used according to Tristan et al. (2003), for protease and toxic shock syndrome toxin-1 genes according to Ote et al. (2011), for A and B enterotoxins according to Park et al. (2011), for C, D and E enterotoxins according to Becker et al. (1998), for enterotoxin H according to Monday & Bohach (1999), while genes for G, I, J, K, L, M, N, O, P and Q enterotoxin were detected using primers described by Bania et al. (2006).

The primers were synthesised by DNA-Gdańsk (Poland). The method described by Tristan et al. (2003) was used to the detection of genes: *cna, fib, fnbA* i *fnbB, ebps, bbp, eno* encoding for the adhesins such as collagen-, fibrinogen-, fibronectin A- and B-, elastin-, bone sialoprotein-, laminin-binding proteins, respectively. The thermal cycling conditions for detection of genes encoding protease (*etA* and *etB*) included predenaturation at 95 °C for 4 min, 35 cycles of denaturation at 95 °C for 0·5 min, primer annealing at 56 °C for 0·5 min and extension at 72 °C for 1 min. The primer annealing at 59 °C was used for the detection of genes coding the remaining proteases (*etD, splA, splE, sspA*). A 5-min extension at 70 °C was performed at the end of the final cycle. The detection of genes coding the enterotoxins A to E was performed using the following cycling conditions: predenaturation at 95 °C for 10 min, 30 cycles of denaturation at 95 °C for 0·5 min, primer annealing at 58 °C for 45 s and extension at 72 °C for 1 min. A 10-min extension at 72 °C was performed at the end of the final cycle. PCR method described by Bania et al. (2006) was used to amplify the genes coding the enterotoxins G to Q, while the gene coding the toxic shock syndrome toxin-1 was detected in accordance with method described by Ote et al. (2011). Amplification of DNA was performed using the Multi Gene II thermal cycler (Labnet International, Inc., USA). The volume of 25 µl of PCR mixture was prepared and contained 2·5 µl of DNA template, 1 × PCR buffer, 0·2 mM each dATP, dCTP, dGTP, and dTTP (Fermentas, Lithuania), 200 nM each of pair of primers, and 1 U of RedTag Genomic DNA polymerase (Sigma-Aldrich, Germany).

The PCR products were analysed by electrophoresis in 1·5% agarose gels stained with ethidium bromide. Molecular size markers (GenoPlast Biochemicals, Poland and Sigma-Aldrich) were also run for product size verification. The gel was electrophoresed in 2 × Tris-borate buffer at 70 V for 1·5 h.

Statistical analyses

Statistics 12 (Analytical Software, Tallahassee, FL) was used for cluster analysis.

Results

The frequency of 30 genes that was investigated by PCR in 124 *Staph. aureus* isolates is shown in Table 1. The results are presented for three groups created according to related genes: the adhesins genes, the proteases genes and the superantigenic toxin genes encoding the enterotoxins and the toxic shock syndrome toxin-1. Among the seven investigated genes encoding proteins that are responsible for adherence of *Staph. aureus* to the host extracellular matrix, the *eno* (91·1%) and *fib* (82·3%) genes, encoding laminin- and fibrinogen binding protein, respectively, were the most frequently identified in tested isolates. The genes encoding fibronectin binding protein were present in 64·5% isolates, among which the *fnbB* gene was detected in 50% isolates. Remaining isolates had the *fnbA*.

The presence of *ebps* gene, encoding elastin binding protein, was detected in 25% of isolates. The *cna* and *bbp* genes, encoding collagen- and bone sialoprotein binding protein, were detected only in two isolates. Cluster analysis of genes encoding adhesins showed that *cna, bbp, fnbA* and *ebps* belonged to one group of genes that were rarely

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Table 1. The prevalence of virulence-associated genes in *Staph. aureus* isolates from cows with subclinical mastitis

Virulence-associated genes	No. (%) of isolates with gene
Adhesins	
cna	2 (1·6)
fib	102 (82·3)
fnbA	18 (14·5)
fnbB	62 (50·0)
ebps	31 (25·0)
bbp	2 (1·6)
eno	113 (91·1)
Proteases	
etA	7 (5·6)
etB	0 (0·0)
etD	11 (8·9)
splA	112 (90·3)
splE	96 (77·4)
sspA	114 (91·9)
Superantigenic toxins	
sea	6 (4·8)
seb	3 (2·4)
sec	8 (6·5)
sed	2 (1·6)
see	0 (0·0)
seg	23 (18·5)
seh	4 (3·2)
sei	26 (21·0)
sej	1 (0·8)
sek	8 (6·5)
sel	4 (3·2)
sem	24 (19·4)
sen	24 (19·4)
seo	17 (13·7)
sep	5 (4·0)
seq	8 (6·5)
tst	3 (2·4)

detected in contrast to the second group of genes (*fib, eno* and *fnbB*) which occurred more frequently in this population of *Staph. aureus* isolates (Fig. 1a). The 17 different combinations of adhesin genes were identified in 124 isolates. Six of them were the most frequent and found in 81·5% of the isolates (Table 2). Three major patterns occurred in 59·7% of the isolates. In all isolates showing these patterns the *fib and eno* genes were detected, furthermore the *fnbB* gene was detected in 46·8%. Additionally, in 14·5% isolates *ebps* gene was detected.

The presence of *etA, etB, etD* genes, encoding exfoliative toxins, was also investigated in *Staph. aureus* isolates from milk of cows with subclinical mastitis. The *etA* and *etD* genes were present in genome of 5·6 and 8·9% isolates, respectively. Whereas, the *etB* gene was not detected in any of the isolates. Of the three investigated serine protease-encoding genes, the *splA* and *sspA* were identified in above 90% isolates. The *splE* gene was present in 77·4% tested *Staph. aureus* (Table 1). Based on cluster analysis, it was found that the genes encoding exfoliative toxins belonged to one cluster rarely observed among isolates, while second cluster comprised genes encoding serine proteases occurred with high frequency, in which *splA* and *sspA* showed very similar frequency of occurrence (Fig. 1b). According to the results, 12 combinations of these six protease genes were identified, with three major patterns found in 82·3% isolates. These patterns differed by presence or the absence of the *splE* and *etD* genes (Table 2).

The genes encoding the enterotoxins were identified in 44 (35·5%) *Staph. aureus* isolates. Of the 16 investigated genes, the *sei* (21%), *sem* (19·4%), *sen* (19·4%), *seg* (18·5%) and *seo* (13·7%) were detected more often than other enterotoxin-coding genes (Table 1) and belonged to one cluster of genes. Remaining toxin genes were rarely detected and were grouped in the separate cluster (Fig. 1c). The *sec, sek* and *seq* genes were identified in 6·5% isolates, while the *sea* and *sep* in 4·8 and 4% isolates, respectively. The *seb* was detected only in 2·4% of isolates, while the *sed* and *sej* genes in 1·6 and 0·8% isolates, respectively. The *see* was absent in all investigated isolates. The *tst* gene, encoding the toxic shock syndrome toxin-1, was harboured by 2·4% isolates. A total of 19 combinations of the superantigenic toxin genes were detected. Three of them (*seg, sei, sem, sen, seo; sec, seg, sei, sem, sen, seo* and *seg, sei, sem, sen*) were the most frequent and found in 16·1% of the isolates. Other isolates positive for superantigenic toxin genes differed in the patterns and harboured from one to seven superantigenic toxin genes (Table 2).

The most common virulotype which included 17·7% of the isolates was virulotype carrying the *splA, splE* and *sspA* genes encoding serine proteases, fibrinogen (*fib*) and laminin (*eno*) binding proteins genes and the *fnbB* gene encoding fibronectin binding protein (Table 3). Besides these genes, in 12·1% of isolates *ebps* gene, encoding elastin binding protein was detected. In 3·2% of isolates besides adhesin genes (*fib, eno, fnbA*) and serine protease genes (*splA, splE, sspA*), the *etD* gene encoding exfoliative toxin and five superantigenic toxin genes (*seg, sei, sem, sen, seo*) were detected. In other 3·2% of isolates only genes encoding serine proteases were present. The other most frequent virulotypes of *Staph. aureus* differed in the presence or absence of the genes encoding different adhesins and proteases.

Discussion

Pathogenic factors of *Staph. aureus* enable this bacteria to attach, colonise, invade and infect the host. In Poland comprehensive data concerning genes encoding adhesins, proteases and enterotoxins in *Staph. aureus* from mastitis cases are limited (Kot et al. 2012a; Jagielski et al. 2014). In our study, we investigated the presence of 30 important virulence genes encoding adhesins, proteases and superantigenic toxins. Among genes encoding adhesins, the *eno* and *fib* genes, encoding adhesion for laminin and fibrinogen, respectively, were ubiquitous in investigated *Staph. aureus* isolates. Fibrinogen binding protein gene is

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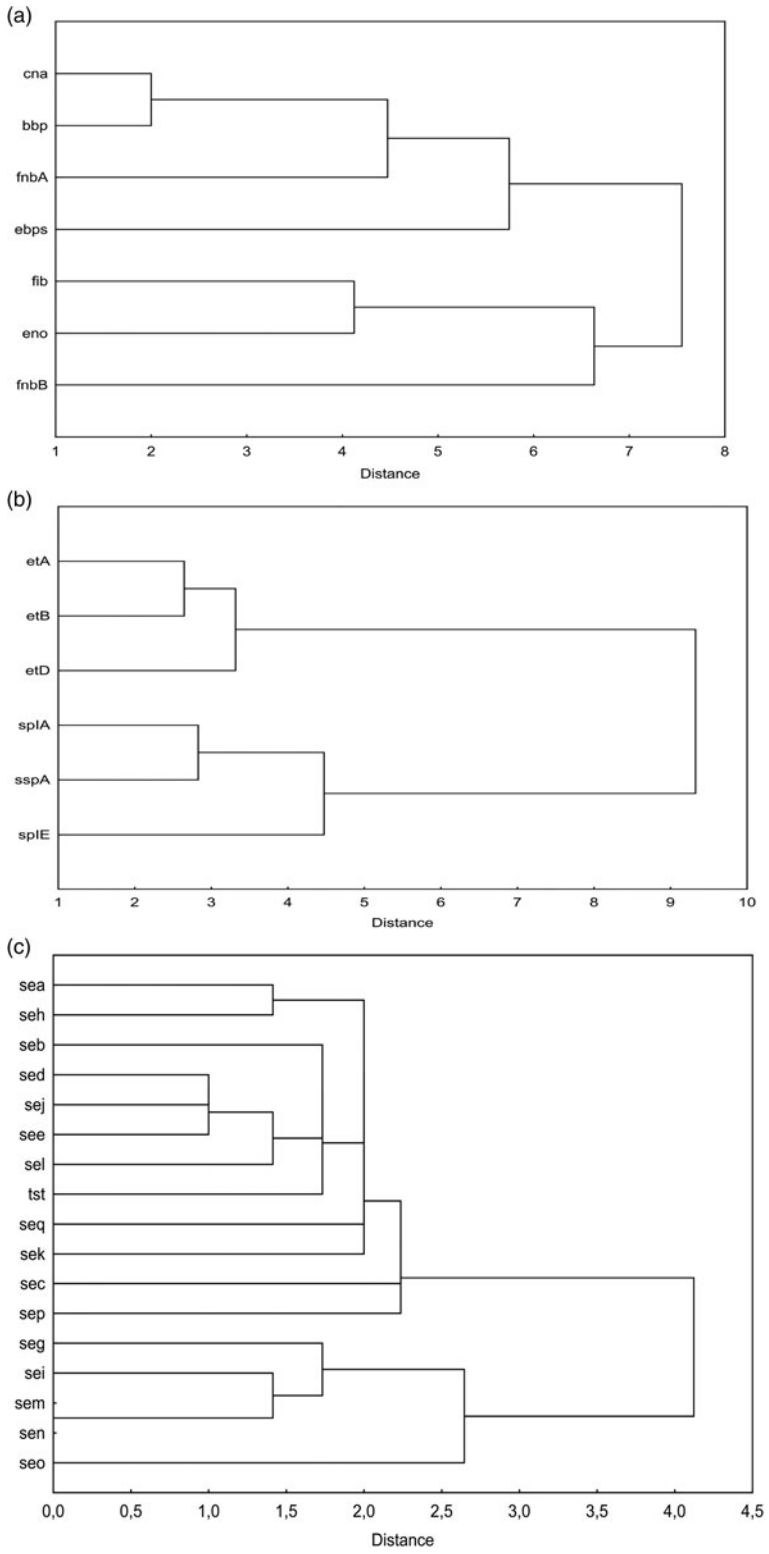

Fig. 1. The groups of genes encoding adhesins (a), proteases (b) and superantigenic toxins (c) in *Staph. aureus* isolated from cows with subclinical mastitis created based on the cluster analysis. (a) – *cna, bbp, fnbA, ebps, fib, eno, fnbB* – genes encoding adhesins such as collagen-, bone sialoprotein-, fibronectin A-, elastin-, fibrinogen-, laminin-, fibronectin B-binding proteins, respectively. (b) – *etA, etB, etD* – genes encoding exfoliative toxins; *splA, sspA, splE* – genes encoding serine proteases. (c) – *sea, seb, sec, sed, see, seg, she, sei, sej, sek, sel, sem, sen, seo, sep, seq* – genes encoding the enterotoxins A to E and genes for G, H, I, J, K, L, M, N, O, P and Q enterotoxin; *tst* – gene encoding the toxic shock syndrome toxin-1.

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Table 2. The most frequent combinations of the adhesin, the protease and superantigenic toxin genes in *Staph. aureus* isolated from cows with subclinical mastitis

Adhesin genes combinations	No. (%) of isolates
fib, eno, fnbB	40 (32·3)
fib, eno, fnbB, ebps	18 (14·5)
fib, eno	16 (12·9)
fib, eno, fnbA	11 (8·9)
fib, eno, ebps	8 (6·5)
eno	8 (6·5)
Protease genes combinations	
splA, splE, sspA	83 (66·9)
splA, sspA	10 (8·1)
splA, splE, sspA, etD	9 (7·3)
Superantigenic toxin genes combinations	
seg, sei, sem, sen, seo	12 (9·7)
sec, seg, sei, sem, sen, seo	4 (3·2)
seg, sei, sem, sen	4 (3·2)
sea, seh, sek, seq	3 (2·4)
tst, sec, seg, sei, sel, sem, sen	2 (1·6)
seb, sek, seq	2 (1·6)
tst, sea, sec, sei, sel, sem, sen	1 (0·8)
sed, sei, sem, sen, seo	1 (0·8)
sea, seh, seq	1 (0·8)
sea, sek	1 (0·8)
seg, sei	1 (0·8)
sed, sej	1 (0·8)
sec, sel	1 (0·8)
sek, seq	1 (0·8)
sep	5 (4·0)
sek	1 (0·8)
seq	1 (0·8)
seb	1 (0·8)
sei	1 (0·8)

widespread among *Staph. aureus* isolated from cows showing mastitis (Singh et al. 2011) but research conducted by Zecconi et al. (2006) did not show statistically significant association between *fib* gene and the presence of subclinical mastitis. The ability to bind laminin correlates with an invasive phenotype of *Staph. aureus* (Carneiro et al. 2004). In our study above 91% *Staph. aureus* isolates showed the presence of gene encoding laminin binding protein, whereas in the research conducted by other authors all *Staph. aureus* isolates involved in human hematogenous infections (Tristan et al. 2003) and intra-mammary infections in cattle and buffaloes (Singh et al. 2011) were positive for this gene. We also investigated the presence of the *fnbA* and *fnbB* genes, encoding fibronectin binding protein. Genes encoding receptor for fibronectin were present in 64·5% of isolates. Haveri et al. (2008) showed that the genes *fnbA* and *fnbB* coexisted in the predominant PFGE types of *Staph. aureus* strains isolated from bovine intramammary infections and extramammary sites in Finland, while only *fnbA* was detected in other, less frequent PFGE types. In our work, *fnbB* was more common than *fnbA* in *Staph. aureus* isolates from subclinical mastitis. The ability of adhesion to fibronectin is important during several steps of the disease process, because fibronectin is a ubiquitous host protein present in soluble form in the blood and in fibrillar form in cellular matrices (Patti et al. 1994). In our study, only 25% of *Staph. aureus* isolates were positive for gene encoding elastin binding protein, while Singh et al. (2011) reported that in different experimental herds of various animals in India, 43% to over 69% *Staph. aureus* isolates from mastitis carried *ebps* gene. Elastin is present in tissues that require elasticity such as the skin, lung, and blood vessels. Elastin-binding protein of *Staph. aureus* may be significant first of all in colonisation of injured tissues abundant in elastin (Downer et al. 2002). In this study, the presence of gene encoding collagen binding protein was detected only in two isolates, what is in agreement with results obtained by others authors who detected this gene in a few of the *Staph. aureus* isolates of bovine origin in India (Singh et al. 2011).

In many studies conducted in other countries, none of *Staph. aureus* isolates from cows with mastitis carried exfoliative toxin genes (Karahan et al. 2009; Piccinini et al. 2010; Singh et al. 2011; Silveira-Filho et al. 2014). In our work, the presence of genes encoding exfoliative toxins A, B and D was investigated, and in 14·5% of isolates *etA* or *etD* genes were detected. The presence *etA* gene in 18·3% *Staph. aureus* isolated from milk of cows with clinical or subclinical mastitis was also detected in Belgium (Ote et al. 2011). This might suggest that the evolutionary precursors to bovine specialist *Staph. aureus* clones were human-associated *Staph. aureus* clones (Herron-Olson et al. 2007).

Staphylococcal proteases are important virulence factors, which can cleave important host proteins, including the heavy chains of all human immunoglobulin classes, plasma proteinase inhibitor and elastin (Potempa et al. 1988). Proteases by degrading bacterial cell surface proteins, such as fibronectin binding protein and protein A allow the transition of *Staph. aureus* cells from an adhesive to an invasive phenotype (Rice et al. 2001). In our study, we investigated the presence of three serine protease-encoding genes. Among them, the *splA* and *sspA* genes were identified in above 90% isolates which is similar to the results obtained by Ote et al. (2011).

The important group of virulence factors of *Staph. aureus* are superantigenic toxins, which might facilitate immunosuppression and would promote the persistence of *Staph. aureus* in cattle, and contribute to chronic infection. Several studies have shown that most *Staph. aureus* harbour one or more enterotoxin genes (Ote et al. 2011; Silveira-Filho et al. 2014). In presented study, in 44 (35·5%) isolates at last one gene encoding superantigenic toxin was detected. Similar prevalence of *Staph. aureus* with enterotoxin genes was shown by Bystroń et al. (2009) who investigated strains from bovine milk from herds localised in the Lower Silesia region in Poland (35%). Frequency of enterotoxin genes differs according to geographical location, and for example in China almost 65%

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Table 3. PCR profiles of the most frequent virulotypes of *Staph. aureus* mastitis isolates

Adhesin genes					Protease genes				Superantigenic toxin genes					No. (%)
fib	*eno*	*fnbA*	*fnbB*	*ebps*	*splA*	*splE*	*sspA*	*etD*	*seg*	*sei*	*sem*	*sen*	*seo*	
+	+	−	+	−	+	+	+	−	−	−	−	−	−	22 (17·7)
+	+	−	+	+	+	+	+	−	−	−	−	−	−	15 (12·1)
−	+	−	−	−	+	+	+	−	−	−	−	−	−	6 (4·8)
+	+	+	−	−	+	+	+	+	+	+	+	+	+	4 (3·2)
+	+	−	+	−	+	−	+	−	−	−	−	−	−	4 (3·2)
+	+	−	−	−	+	+	+	−	−	−	−	−	−	4 (3·2)
−	−	−	−	−	+	+	+	−	−	−	−	−	−	4 (3·2)

of the isolates from bovine mastitis cases harboured at last one enterotoxin gene (Wang et al. 2009), while in Brazil 68·4% *Staph. aureus* from milk were positive for one or more enterotoxin gene (Rall et al. 2008). The most frequently observed enterotoxin genes in *Staph. aureus* from our collection were *sei, sem, sen, seg* and *seo* and comprised one cluster in terms of frequency. Our results are consistent with reports of other authors, who showed that the newly described enterotoxin genes are predominant among *Staph. aureus* isolated from food, various animal species and food poisoning cases in humans (Rosec & Gigaud, 2002; Smyth et al. 2005; Bania et al. 2006). The *sei* and *seg* genes were also detected in high frequency among *Staph. aureus* isolates from bovine mastitis in Switzerland, Indonesia and China (Akineden et al. 2001; Stephan et al. 2001; Wang et al. 2009). Many studies show that the *sei* and *seg* genes occur together in *Staph. aureus* (Zschöck et al. 2005; Bania et al. 2006; Silveira-Filho et al. 2014) which is corresponding with our results. Jarraud et al. (2001) found that the *sei* and *seg* genes coexist with three other genes: *sem, sen, seo* and sometimes with *seu* in a common genetic element, called the enterotoxin gene cluster (*egc*), and this cluster is located on the genomic island Type II vSaβ. In our study the *egc* was detected in 26 isolates but complete cluster was occurred only in 16 isolates. The presence of variants or incomplete clusters of genes encoding enterotoxin was observed in *Staph. aureus* isolated from milk of cows with clinical and subclinical mastitis in Belgium (Ote et al. 2011). We detected isolate which had only *sei* gene, although we used the primers designed by Bania et al. (2006) targeting the conserved regions of *sei* and *seg*. The *Staph. aureus* strains with *sei* or *seg* alone were also detected by other researchers (Omoe et al. 2003; Zecconi et al. 2006; Karahan et al. 2009). In our results, the classical enterotoxin genes were rarely detected which confirmed the results obtained by other authors, who showed that these genes were absent or seldom detected in *Staph. aureus* isolated from cows with mastitis (Zecconi et al. 2006; Bystroń et al. 2009; Karahan et al. 2009; Piccinini et al. 2010). In this study, the *sea* gene was detected in only 4·8% of the isolates which is in concordance with the results obtained by Ote et al. (2011) who found this gene in 5·2% of isolates associated with bovine mastitis. The presence of *sea* gene in genome of *Staph. aureus* was detected with different frequency in studies conducted by other authors. Fournier et al. (2008) reported that 30% of *Staph. aureus* strains from milk of cows with intramammary infection showed *sea* gene, while it was absent in strains from milk of cows with mastitis investigated by other researchers (Srinivasan et al. 2006; Karahan et al. 2009). The observed discrepancy may be due to irregular geographical distribution of bacteriophages the genomes of which contain *sea* gene, therefore, the frequency of infected *Staph. aureus* is different or even lacking (Le Loir et al. 2003).

The results by other authors also showed, as in our study, that *seb* and *see* genes were absent or present in a very few isolates (Fournier et al. 2008; Bystroń et al. 2009; Ote et al. 2011). In this study, we also investigated the presence of *tst* gene and this gene was detected in 3 *Staph. aureus* isolates. The *Staph. aureus* isolates negative for *tst* genes were detected among isolates from dairy herds in Italy (Piccinini et al. 2010) and in the Lower Silesia in Poland (Bystroń et al. 2009). Only three *Staph. aureus* from bovine subclinical mastitis in Turkey were positive for *tst* (Karahan et al. 2009). In our study, among isolates with *tst* genes, two also carried *sec, seg, sei, sel, sem* and *sen* genes and one had *tst, sea, sec, sei, sel, sem, sen* genotype. Zschöck et al. (2004) reported the presence of a positive correlation between *sec-tst*. In this study, *sec* gene was detected in all *tst*-positive isolates.

In conclusion the genes encoding laminin-, fibrinogen- and fibronectin binding proteins were the most commonly identified among the genes encoding adhesins. The genes encoding serine proteases also were ubiquitous. Among superantigenic toxins genes, the newly defined enterotoxin genes such as *sei, sem, sen, seg* and *seo* were the most frequently detected. Investigation of virulence gene profiles of *Staph. aureus* revealed a variation in the prevalence of different adhesin and toxin genes in the isolates associated with subclinical bovine mastitis. The presence of isolates with genes encoding toxins involved in human infections in the milk of cows with mastitis is a potential danger for human health. The identification of pathogenic factors of *Staph. aureus* isolates allows a more detailed characterisation of this pathogen, which is important for evaluation of pathogen transmission rate, disease development rate and its severity. Therefore, analysis of potential virulence of

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Staph. aureus provides information important for choice of appropriate methods of prophylaxis and therapy of mastitis.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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