Staphylococcus aureus is among the top five pathogens responsible for acquired foodborne illnesses in Europe (7). Staphylococcal food poisoning (SFP) develops after ingestion of products containing staphylococcal enterotoxins (SEs) and is manifested by vomiting, frequently accompanied by gastroenteritis. SEs are a group of heat-stable, pepsin-resistant exotoxins belonging to a large family of pyrogenic toxin superantigens (PTSAgs) encoded on phage, pathogenicity islands, chromosome, or plasmids (1, 5). In addition to the initially discovered SEA to SEE, known as classical SEs, a number of new enterotoxins have later been described (28, 34). To date, twenty-four members of the SE family have been identified. Some of the new toxins, namely SEG, SEH, SEI, and SER, have been shown to have emetic effect (25, 28, 32). Emetic effect of another new toxin, SELP, has been demonstrated in a small rodent, but not confirmed in a primate model. Thus, despite its potential emetic activity, SELP still cannot be classified as a true SE (27). It is estimated that 5-10% of SFP cases in which none of SEA-SEE enterotoxins were detected can be attributed to other emetic SEs (11).

Genetic background can affect the repertoire of mobile genetic elements in S. aureus (23). Some S. aureus genotypes can occur in both animals and humans (12, 24). However, it seems that the association between certain enterotoxin genes and specific staphylococcal clones may differ in S. aureus isolates of human and animal origin (12, 33).

The aim of this study was to determine the incidence of genes encoding emetic staphylococcal enterotoxins (SEs) in S. aureus isolates from pork and pigs, and to demonstrate the connection between the enterotoxigenic potential of S. aureus and its genetic background.

Materials and methods: S. aureus isolates from pork (45 isolates) and pigs (45 isolates), representing various clonal complexes, were tested for the presence of emetic SEs genes.

Results and discussion: Thirty-four of the 45 S. aureus isolates (75%) derived from pork were shown to harbor genes encoding emetic SEs. Among 45 pig-derived S. aureus isolates, SE genes were detected in 28 isolates (62%). Fifty-five percent of potentially enterotoxigenic staphylococci carried genes encoding classical toxins (SEA-SEE), whereas 28 isolates (45%) harbored exclusively genes encoding new emetic SEs. The most prevalent (82%) classical enterotoxin gene was seb, whereas seg and sei genes dominated (82%) among isolates harboring genes encoding other emetic toxins. Seventeen of 23 S. aureus isolates assigned to the CC15 clonal complex were found to harbor the seb gene. Ten of 15 CC7 isolates contained the selp gene. Isolates harboring seg and sei genes dominated in CC30 (81%) and CC9 clones (76%). Four isolates assigned to CC398 were shown to harbor enterotoxin genes, such as seb, sed, seg, sei, and ser. Our results indicate a high incidence of enterotoxigenic S. aureus isolates harboring genes encoding other emetic SEs in pork and pigs. In most of the pig- and pork-derived isolates studied here, genotype-enterotoxin association was similar to that known from human S. aureus isolates. This is the first report on SE genes in S. aureus CC398 genetic background in Poland, and probably also in Europe.

Keywords: Staphylococcus aureus, staphylococcal enterotoxin genes, genotype, pig, pork
isolates) were used in this study. The animal-derived strains were isolated from pigs’ nasal swabs in slaughterhouses, and the meat-derived isolates were obtained from retail pork in the Lower Silesia region (Poland). The isolates were identified as *Staphylococcus aureus* on the basis of their ability to coagulate rabbit plasma and clumping-factor production. All isolates were screened by PCR with primers for the *S. aureus* specific *nuc* gene as described by Martin et al. (22). Reference *S. aureus* strain ATCC 29213 served as a control. spa genotypes of all the isolates and their assignment to clonal complexes (CC) were determined previously (18, 19) according to the method described by Krupa et al. (17).

Preparation of bacterial DNA. Two milliliters of a bacterial cell suspension from an overnight culture grown in brain-heart infusion broth were centrifuged for 5 min at 12,000 × g, and suspended in 100 µl of 100 mM Tris-HCl buffer, pH 7.4, containing 10 µg of lysostaphin (Sigma-Aldrich, Poznan, Poland). After 30 min of incubation at 37°C, 10 µl of 10% SDS was added, and the sample was incubated for another 30 min at 37°C. Two hundred µl of 5 M guanidine hydrochloride was added, and the sample was mixed by vortexing and incubated at room temperature for 10 min. DNA was extracted with phenol and chloroform, ethanol-precipitated, and dissolved in water.

Detection of enterotoxin genes by PCR. The detection of genes coding for the enterotoxins SEA to SEE was performed by the method described by Sharma et al. (31). The primers for *seg, seh, sei,* and *selp* and *ser* detection were designed on the basis of the alignment of published sequences, as described in our previous works (2, 3). The *ser* gene was detected with the following primers: SER-for (number of isolates) 5′-GCGGCTGACCTGATCCTC-3′, SER-rev (number of isolates) 5′-TTTGCGTTGTGCCTTTG-3′, and SER+rev (number of isolates) 5′-ATCCTTTCTGACGCCTGG-3′.

Enterotoxin genes were detected by four PCR reaction mixtures. The first contained primers for *sea, sec,* and *see.* The second contained primers for *seb* and *sed,* the third contained primers for *seg, seh, sei,* and *selp,* and the fourth contained primers for *ser.* Five enterotoxigenic reference strains were used as positive controls: FR1137 (*sec, seg, seh, sei*), FR1913 (*sea, sec, see*), CCM5757 (*seb*), FRI 1151m (*sed, ser*) (34), and A900322 (*seg, sei, selp*). The PCR was performed in a total volume of 25 µl. In each case, the reaction mixture contained 1 × polymerase buffer with 50 mM KCl, 10 mM Tris-HCl, 4 mM MgCl₂, 0.2 mM of each dNTP, 30 pmole of each primer (Institute of Biochemistry and Biophysics, Warsaw, Poland), 1 µl of DNA solution, and 1 U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Thirty-five cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 1.5 min were performed with a MJMini thermal cycler (BioRad, Hercules, USA). Each PCR was run with a mix of DNA from the corresponding reference strains as positive controls. Ten-µl aliquots of PCR products were resolved on 2% agarose gel at 100 V and documented with a GelDoc XR documentation system (BioRad, Hercules, USA).

**Results and discussion**

Thirty-four of the 45 *S. aureus* isolates (75%) derived from pork were shown to harbor genes encoding emetic SEs. In 23 isolates, *sea-see* genes were detected, whereas 11 isolates contained other SE genes. Specifically, the *seb* gene was found in 20 isolates, *selp* in 8 isolates, *seg* and *sei* in 5, *sec* in 4, and *seh* in 2 isolates. Ten isolates harbored two or three SE genes (Tab. 1).

Among 45 pig-derived *S. aureus* isolates, SE genes were detected in 28 isolates (62%). In 11 isolates, *sea-see* genes were found, and 17 isolates contained other SE genes. In 18 isolates, *seg and sei* genes were found, *seb* was detected in 8, *selp* in 6, *sec* in 3, *sed* and *ser* in 1 isolate. Nineteen isolates harbored two or three SE genes (Tab. 1).

Seventeen of 23 *S. aureus* isolates assigned to the CC15 clonal complex were found to harbor the *seb* gene. Ten of 15 CC7 isolates included the *selp* gene.

Tab. 1. Incidence of staphylococcal enterotoxin genes (SEs) in pig- and pork-derived *S. aureus* isolates representing various clonal complexes.

<table>
<thead>
<tr>
<th>Pigs (number of enterotoxigenic isolates/number of isolates)</th>
<th>Distribution of SEs in <em>S. aureus</em> isolates (number of enterotoxigenic isolates/number of isolates)</th>
<th>Pork (number of enterotoxigenic isolates/number of isolates)</th>
<th>Clonal complex (number of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no isolates (0/0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>selp</em> (4/7)</td>
<td></td>
<td></td>
<td>CC1 (3)</td>
</tr>
<tr>
<td>no isolates (0/0)</td>
<td></td>
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<tr>
<td><em>seb</em> (1/11); <em>seg + sei</em> (2/11); <em>seb + seg + sei</em> (4/11); <em>sec + seg + sei</em> (2/11)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>no isolates (0/0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>selp</em> (2/4)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>no isolates (0/0)</td>
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<tr>
<td><em>seg + sei</em> (8/10)</td>
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</tr>
<tr>
<td><em>sec</em> (1/1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>no isolates (0/0)</td>
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<td></td>
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<tr>
<td>no isolates (0/0)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>seb</em> (1/12); <em>seb + sed + ser</em> (1/12); <em>seb + seg + sei</em> (1/12); <em>seg + sei</em> (1/12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total: 28/45</td>
<td>Total: 34/45</td>
<td>Total: 90</td>
<td></td>
</tr>
</tbody>
</table>
Isolates harboring seg and sei genes dominated in CC30 (81%) and CC9 clones (76%). Four isolates assigned to CC398 were shown to harbor enterotoxin genes, such as seb, sed, seg, sei, and ser (Tab. 1).

Relatively little is known on the enterotoxigenic potential of livestock-associated S. aureus. In this study, 69% of 90 S. aureus isolates examined harbored genes encoding emetic enterotoxins. Fifty-five percent of potentially enterotoxigenic staphylococci carried genes encoding classical toxins (SEA-SEE), whereas 28 isolates (45%) harbored exclusively genes encoding new emetic SEs. The most prevalent (82%) classical enterotoxin gene was seb, whereas seg and sei genes dominated (82%) among isolates harboring genes encoding other emetic toxins. Similarly, among enterotoxigenic S. aureus from pigs in Switzerland, isolates containing seg and sei genes were dominant (63%), whereas classical toxin genes, represented by the sec gene, were detected in only 2% of the isolates (26). The seg and sei genes were also the most prevalent enterotoxin genes among S. aureus isolates from retail meat, including pork, in Korea and the United States (14, 29). These two genes are usually detected together in S. aureus (15). As found by Jarraud et al. (15), seg and sei, together with selm, seln, and selo, are linked in an operon called the enterotoxin gene cluster (egt). Evidence for SEG and SEI expression is indirect only, so their role in SFP remains unclear (13).

Certain S. aureus genotypes were shown to be associated with a specific enterotoxin gene repertoire (23). In most of the pig and pork-derived isolates studied here, genotype/enterotoxin association was similar to that known from human S. aureus isolates. This applies especially to seb and seh genes, which were frequently found in CC1 (20), to selp, which was found in CC7, as well as to sec and selp, prevailing in CC12 (12). The seg and sei genes, belonging to egc, have already been associated with CC9, CC30, CC25, and CC45 background in human, food, and bovine S. aureus isolates (8, 12, 30). According to our data, seg and sei also dominate in the abovementioned genotypes of pig-associated S. aureus. The lack of enterotoxin genes is a characteristic trait of bovine CC97 and CC101 S. aureus isolates (30). According to our data, S. aureus CC97 and CC101 from pigs can also constitute unfavorable background for SE incorporation.

Relatively little is known about the SE gene content in the livestock-associated CC398 lineage. Studies on European isolates belonging to CC398 report the absence of SE genes from this genetic background (3, 8-10, 16, 21, 24, 30). On the other hand, 33% of pig-associated S. aureus population studied here was found to be enterotoxigenic. Liu et al. (20) recently reported on the incidence of SEs in S. aureus CC398 of human origin in China. The seb, seg and sei genes detected in Chinese CC398 human isolates were also found in our CC398 pig-associated isolates.

According to the European legislation on food safety, SEA-SEE are the only SEs routinely detected in food (6). Our results indicate a high incidence of enterotoxigenic S. aureus isolates harboring genes encoding other emetic SEs in pork and pigs, which implies the need for new methods of tracking currently underestimated food hazards. The present study is the first report on SE genes in S. aureus CC398 genetic background in Poland, and probably also in Europe. This indicates that a typical animal-associated S. aureus clone regarded so far as neutral for food safety may in fact pose a potential risk for consumers.

References


