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ORIGINAL ARTICLE

Detection of some phenotypic and genotypic characteristics of *Staphylococcus aureus* isolated from food items in the Czech Republic

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Abstract *Staphylococcus aureus* is an important foodborne pathogen and can produce a wide range enterotoxins, which contribute to food poisoning. The aim of this study was to interrogate foodborne strains of *S. aureus* for phenotypic and genotypic characteristics. Strains were screened for enterotoxins, hemolysins and antimicrobial resistance, and the genetic relationship between strains was described after pulsed-field gel electrophoresis (PFGE) analysis. Of the *S. aureus* strains, 82.8 % (n=93) harboured one or more of the following enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej*); 39.8 % of strains demonstrated *se* genes and 43 % carried from two to five *se* genes, while 17.2 % of the strains possessed none of the genes examined. The most commonly detected toxin genes were *sea*, *seb*, *sec* and *seg*. The presence of genes coding for antibiotic resistance such as *blaZ, vanA, vanB* and *mecA* was investigated by polymerase chain reaction (PCR). Seventy-two strains carrying the *blaZ* gene exhibited phenotypic resistance to ampicillin and penicillin. Ten strains (10.75 %) carried the *mecA* gene and correspondingly demonstrated resistance to oxacillin. The presence of vancomycin resistance genes, *vanA* and *vanB*, was not detected. Genotypic subtyping was performed using PFGE with *Sma*I restriction enzyme. The genetic relationships between enterotoxin harboring strains and non-enterotoxigenic strains were explored. Twenty-four different pulsotypes were generated from 93 food isolates with a similarity level of 88 %.

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Introduction

Staphylococcus aureus strains can produce a wide range of extracellular toxic proteins while growing or occurring in food. This may result in outbreaks of staphylococcal food poisoning (SFP) in humans and animals (Jablonski and Bohach 2001). To date, 23 different types of staphylococcal enterotoxins (SE) have been identified and divided into two groups according to their demonstrated emetic activity: classical SE and new SE. The members of classical enterotoxins are SEA, SEB, SEC (with the SEC1, SEC2, SEC3, SEC-ovine and SEC-bovine variants). SED and SEE are the most frequent cause of SFP. SEA is the most common enterotoxin recovered from food-poisoning outbreaks in the EU (53.6 % of all outbreaks), followed by SED (37.5 %) and SEB (10 %) (Kérouanton et al. 2007). In recent years, SEF (TSST), SEG, SEH, SEI, SElJ, SElK, SElL, SElM, SElN, SElO, SElP, SElQ, SER, SE, SET, SElU, SElUv (SEW), SElV and SElX have been identified as new SE and SE-like toxins (Omoe et al. 2004; Bania et al. 2006; Argudín et al. 2010; Schelin et al. 2011). Several of the more recent enterotoxins isolated from food poisoning cases include SEG, SEH, SEI and SElJ. SE are resistant to inactivation by gastrointestinal proteases such as pepsin, as well as by heat. Heat stability, unlike the producer organism, is one of the most resilient properties of SE in terms of food safety (Balaban and Rasooly 2000; Peles et al. 2007; Schelin et al. 2011).

A range of typing methods have been employed to characterise *S. aureus* isolates. Phenotypic and genotypic methods have gradually been supplemented or replaced with genotyping methods. Polymerase chain reaction (PCR) is currently used as a simple and robust technique for detecting

enterotoxigenic strains and resistance in staphylococcal strains (Asperger and Zangerl 2003). Pulsed-field gel electrophoresis (PFGE) using *Sma*I endonuclease is one classical, discriminatory method for the molecular characterisation of *S. aureus* genotypes (Weller 2000). Unfortunately, isolates of the ST398 clone are nontypeable (NT) by *Sma*I-PFGE. When PFGE was performed on ST398 isolates, no banding patterns were generated. This is a result of the action of an indirectly revealed DNA methyltransferase that modifies the consensus sequence $C^{m}CNGG$ at the second cytosine. Therefore, ST398 isolates are referred to as PFGE non-typeable (NT *Sma*I)-methicillin-resistant *S. aureus* (MRSA) (Argudín et al. 2009).

The aims of this study were to phenotypically and genotypically characterise foodborne *S. aureus* strains, to determine the frequency of SE genes (*sea, seb, sec, sed, see, seg, seh, sei, sej*), and to investigate the genetic-relatedness of enterotoxigenic and non-enterotoxigenic isolates of foodborne *S. aureus* isolates by PFGE.

Material and methods

Bacterial strains and identification

The study population comprised a total of 99 *S. aureus* strains, consisting of six reference strains and 93 strains isolated from food samples. All isolates were obtained from the National Institute of Public Health in Brno, National Reference laboratory in Prague, Czech Collection of Microorganisms (CCM) and other isolates that were previously isolated in our laboratory. The *S. aureus* strains were isolated the following food products: milk (n=59); meat products (n=7); fish (n=9); confectionery products (n=9); sausage and ham (n=5); and other miscellaneous food products (n=4) (Table 1). The geographical sources of these isolates were as follows: Brno (n=26), Praha (n=15), Příšovice (n=5), Ostrava (n=2), Ústí nad Labem (n=2), Újezd u Brna (n=10), Opočno (n=6) and other districts (n=27). *Staphylococcus aureus* strains were detected on Baird Parker agar with Egg Yolk Tellurite Emulsion (BP, Merck, Germany) incubated at 37 °C for 24–48 h and then examined for coagulase activity using rabbit plasma according to ISO 1999a and ISO 1999b. Coagulase-positive staphylococci were confirmed as *S. aureus* using standard microbiological procedures, e.g., Gram staining, catalase and oxidase reactions. Strains were also streaked on blood agar plates for detection of haemolytic activity. *Staphylococcus aureus* phosphatase activity was tested using selective chromogenic culture medium SaSelect ™ Medium (Bio-Rad, USA). The strain collection was stored at −80 °C in Tryptone Soy Broth (TSB, Merck, Germany) and glycerol for further characterization.

DNA Extraction and identification by PCR

Total genomic DNA was extracted as previously described by Valihrach et al. (2009). Working cultures were prepared in 5 ml TSB and incubated at 37 °C for 24 h. After incubation, 1 ml of the culture, approximately $1–5\times10^{9}$ CFU/ml bacterial cells, was centrifuged at 12,000×*g* for 10 min, and the supernatant was removed. The pellet was resuspended in 200 μl of deionised water (dH_2O), heated at 100 °C for 20 min. and centrifuged again at 12,000×*g* for 6 min. The supernatant was transferred to a new tube and used as the DNA template for PCR assay. After extraction, DNA concentration was measured using a nanophotometer (İmplen, Germany). DNA preparations were kept at −20 °C prior to further testing.

The specific primers Sa442-1 (5′-AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG-3′) and Sa442-2 (5′-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3′) were used for strain identification by multiplex PCR according Martineau et al. (1998). Confirmation of the target 16S rRNA (*Staphylococcus* genus specific) and Sa442 (*S. aureus* species specific) was performed on all strains.

Detection of virulence and resistance genes

The detection of nine staphylococcal enterotoxin genes (*sea, seb-sec, sec, sed, see, seg, seh, sei* and *sej*) in staphylococcal isolates was determined according to Monday and Bohach (1999) and Løvseth et al. (2004). Primers used to amplify antibiotic resistance genes (*mecA, blaZ, vanA* and *vanB*) by Murakami et al. (1991) and Spanu et al. (2012) were employed. Amplification of the target 16S rRNA gene was included as an internal control. Different strains of *S. aureus* were supplied by Prof. Jiří Doškař (Brno, Czech Republic): MW2 for *sea*, *sec*, *seh*, *mecA, blaZ* genes; Mu50 for *sea*, *seb*, *sec*, *seg*, *sei, mecA* genes; FRI 137 for *sec, seg, seh, sei* genes; FRI361 for *sec, sed, seg, sei, see, selj* genes; and as a negative control we used non-template. DNA was amplified with the following thermal settings: the initial denaturation (10 min, 95 °C); 15 cycles of annealing at 68 °C (95 °C, 1 min; 68 °C, 45 s; 72 °C, 1 min), 20 cycles of annealing at 64 °C (95 °C, 1 min; 64 °C, 45 s; 72 °C, 1 min); final extension (72 °C, 10 min). Amplification was performed in a T-Gradient Thermocycler Biometra (Whatman, Germany) using a *Taq* DNA polymerase (Promega, Germany). Ten μl of PCR products were separated in 2 % agarose gel in 1× TBE (Tris-Borate-EDTA, Eppendorf, Germany) as described by Sambrook and Russell (2001), stained with ethidium bromide (0.5 mg/ml) at 100 V for 60 min and visualized on a UV transilluminator (Vilber Lourmat, France). PCR experiments were performed in duplicate for test and controls strains.

Table 1 Phenotypic and genotypic properties of all *S. aureus* strains obtained from different food samples

Strains	Origin	Antibiogram profile		Coagulase test	Hemolysis	Colour in chromogenic culture medium	Enterotoxin and antibiotic resistance genes
		TET	AMP				
STA 038	Raw cow's milk	S	S	Positive	β	Orange	*sec*
STA 039	Raw cow's milk	S	S	Positive	α	Pink	*sec*
STA 057	Feta cheese	S	R	Positive	β	Orange	*seh, blaZ*
STA 040	Raw cow's milk	S	S	Positive	β	Orange	*sec*
STA 054	Confectionery product	S	R	Positive	β	Orange	*sea, seh, blaZ*
STA 065	Milk product	S	R	Positive	α–β	Orange	*blaZ*
STA 024	Milk product	S	R	Positive	β	Pink- Orange	*sed, blaZ*
STA 025	Milk product	S	R	Positive	β	Orange	*sed, blaZ*
STA 033	Raw milk	S	R	Positive	α–β	Pink	*sec, see, sea, seb, blaZ*
STA 034	Raw milk	S	R	Positive	α–β	Orange	*sec, blaZ*
STA 066	Seafood (fish)	S	R	Positive	α–β	Pink- Orange	*seb, seh, blaZ*
STA 008	Fish product	R	R	Positive	α	Orange	*sec, seg, sed, sej, blaZ*
STA 052	Pork ham	S	R	Positive	α–β	Pink	*sec, sei, sea, blaZ*
STA 064	Pork ham	S	R	Positive	β	Orange	*sea, seb, sed, sej, blaZ*
STA 050	Fish product	S	S	Positive	α	Orange	*sec, sea*
STA 009	Raw cow's milk	S	R	Positive	α–β	Pink	*sec, seg, sed, sej, blaZ*
STA 072	Milk product	S	R	Positive	β	Orange	*seh, sec, blaZ*
STA 048	Raw cow's milk	R	R	Positive	α–β	Pink	*mecA, blaZ*
STA 080	Milk product	S	R	Positive	α–β	Pink	*seb, blaZ*
STA 053	Raw milk	S	R	Positive	α–β	Pink	*sec, seg*
STA 006	Raw cow's milk	S	R	Positive	Weak	Orange	*sec, sea, blaZ*
STA 023	Milk product	S	R	Positive	α–β	Pink- Orange	*blaZ*
STA 041	Raw milk	S	R	Positive	Weak	Orange	*sec, blaZ*
STA 090	Minced meat	S	R	Positive	α	Orange	*sed, sej, blaZ*
STA 062	Raw milk	S	R	Positive	β	Orange	*sed, sej, blaZ*
STA 071	Meat product	S	R	Positive	β	Pink	*Sej, blaZ*
STA 073	Raw milk	S	R	Positive	β	Pink	*blaZ*
STA 091	Cow's milk	S	R	Positive	α–β	Pink	*blaZ*
STA 026	Milk product	S	R	Positive	α–β	Orange	*seb, blaZ*
STA 018	Sheep's milk	S	R	Positive	Weak	Orange	*sec, blaZ*
STA 029	Sheep's milk	S	R	Positive	α	Pink	*sec, mecA, blaZ*
STA 030	Sheep's milk	S	S	Positive	α	Orange	*sec*
STA 028	Raw goat's milk	S	R	Positive	Weak	Pink	*sec, blaZ*
STA 027	Milk product	S	R	Positive	α	Orange	*sed, mecA, blaZ*
STA 088	Raw milk	S	R	Positive	α–β	Orange	*blaZ*
STA 063	Seafood (fish)	S	R	Positive	α	Orange	*sea, blaZ*
STA 079	Raw milk	R	R	Positive	α–β	Pink	*seg, seb, blaZ*
STA 031	Sheep's milk	S	R	Positive	α–β	Orange	*sec, blaZ*
MW2[a]	Human	S	R	Positive	β	Orange	*sea, sec, seg, seh, sek, seq, sel, mecA, blaZ*
STA 093	Meat product	S	R	Positive	β	Orange	*sea, blaZ*
STA 020	Milk product	S	R	Positive	β	Orange	*blaZ*
STA 021	Milk product	S	R	Positive	β	Orange	*sec, blaZ*
STA 084	Milk product	S	S	Positive	α–β	Pink	*sec, sed, sea*
STA 085	Raw cow's milk	S	R	Positive	α–β	Orange	*sec, blaZ*
STA 016	Sausage	S	S	Positive	α–β	Orange	*sec*

Table 1 (continued)

Strains	Origin	Antibiogram profile		Coagulase test	Hemolysis	Colour in chromogenic culture medium	Enterotoxin and antibiotic resistance genes
		TET	AMP				
STA 044	Raw cow's milk	S	S	Positive	α	Pink	*sec, seb, sea*
STA 043	Raw milk	S	S	Positive	β	Orange	*sec, sea*
STA 045	Raw cow's milk	S	S	Positive	α	Pink	*sec*
STA 012	Pork ham	S	R	Positive	α	Pink	*sec, blaZ*
STA 014	Spinach	S	R	Positive	α–β	Orange	*sec, blaZ*
STA 058	Pork ham	S	S	Positive	β	Orange	*seb*
STA 001	Fish product	S	S	Positive	α–β	Pink	*sec*
STA 042	Raw cow's milk	S	R	Positive	Weak	Pink	*sec, sei, seg, blaZ*
STA 056	Confectionery product	S	R	Positive	β	Orange	*sed, sei, seg, mecA, blaZ*
STA 089	Confectionery product	S	S	Positive	α	Pink	*seb*
STA 074	Cereal porridge	S	S	Positive	α	Orange	*seh, sei, seg, sea*
CCM 3953 (ATCC 25923)[b]	Clinical isolate	S	S	Positive	β	Pink	*sea,seg,sei*
STA 055	Meat product (salami)	S	R	Positive	β	Pink	*seb, sej, sed, blaZ*
STA 083	Raw cow's milk	S	R	Positive	β	Orange	*sei, seg, blaZ*
STA 010	Hard Edam cheese	R	R	Positive	α–β	Pink	*sec, blaZ*
STA 077	Raw milk	S	R	Positive	α–β	Pink	*sec, sei, blaZ*
STA 035	Frozen cream	S	R	Positive	β	Pink	*sec, sei, seg, blaZ*
STA 036	Raw milk	S	R	Positive	α–β	Orange	*sec, blaZ*
STA 046	Raw cow's milk	R	R	Positive	α–β	Orange	*sei, seg, blaZ*
STA 047	Raw cow's milk	S	R	Positive	β	Orange	*blaZ*
STA 081	Confectionery product	S	R	Positive	α	Orange	*sec, sea, blaZ*
STA 003	Raw cow's milk	S	R	Positive	β	Orange	*mecA, blaZ*
STA 061	Raw milk	S	R	Positive	α–β	Orange	*seb, sei, seg, mecA, blaZ*
STA 013	Poultry sausage	S	R	Positive	α–β	Orange	*sec, sea, sei, seg, blaZ*
STA 032	Sheep's milk	S	R	Positive	α–β	Pink	*sec, blaZ*
STA 007	Raw cow's milk	R	R	Positive	α–β	Pink	*sec, mecA, blaZ*
STA 060	Raw cow's milk	S	R	Positive	β	Orange	*sed, sei, seg, sej, mecA, blaZ*
STA 075	Meat product	S	R	Positive	α–β	Orange	*blaZ*
FRI 361[c]	Cooked chicken	S	R	Positive	α–β	Orange	*sec, seg, sed, sej, sei, sel*
STA 017	Raw cow's milk	S	R	Positive	Weak	Orange	*sec, sei, seg, blaZ*
STA 019	Raw cow's milk	S	R	Positive	α	Orange	*sec, sei, seg*
STA 004	Confectionery product	R	R	Positive	β	Pink	*seg, sei, sec, blaZ*
STA 015	Confectionery product	S	R	Positive	α–β	Orange	*sec, sei, seg, blaZ*
STA 049	Raw cow's milk	R	R	Positive	β	Orange	*blaZ*
NCTC 8325[d]	Human (sepsis patient)	S	S	Positive	β	Pink	*sea*
STA 076	Fish product	S	R	Positive	Weak	Pink	*blaZ*
STA 078	Meat product	S	S	Positive	α–β	Orange	*sea, seh*
STA 022	Milk product	S	R	Positive	β	Orange	*blaZ*
STA 059	Seafood (fish)	S	R	Positive	α–β	Pink	*sea, seb*
STA 082	Eggs	R	R	Positive	α	Orange	*seg, sec, sei, sea, blaZ*
STA 067	Meat product	S	R	Positive	α–β	Orange	*blaZ*
STA 069	Raw milk	S	R	Positive	Weak	Pink- Orange	*blaZ*
STA 051	Raw cow's milk	S	R	Positive	Weak	Orange	*sec, blaZ*
STA 005	Fish fillets	S	R	Positive	α–β	Orange	*sec, mecA, blaZ*
STA 086	Raw cow's milk	R	R	Positive	Weak	Pink	*sec, seb, sed, mecA, blaZ*
STA 002	Raw cow's milk	S	S	Positive	α–β	Pink	none

Table 1 (continued)

Strains	Origin	Antibiogram profile		Coagulase test	Hemolysis	Colour in chromogenic culture medium	Enterotoxin and antibiotic resistance genes
		TET	AMP				
FRI 137[e]	Leg abscess	R	S	Positive	β	Pink	*sec, seg, seh, sei, sel*
STA 037	Raw cow's milk	S	R	Positive	α–β	Orange	*sec, blaZ*
STA 011	Fish fillets	S	R	Positive	α	Orange	*sec, sea, see, blaZ*
STA 087	Pork ham	R	R	Positive	α–β	Pink	*sec*
STA 070	Raw milk	R	R	Positive	α–β	Orange	*sei, seg, blaZ*
STA 092	Chicken Tetrazzini	S	S	Positive	α–β	Pink	*see*
STA 068	Milk product	S	R	Positive	Weak	Pink- Orange	*sec, sei, seg, blaZ*

TET Tetracycline; *AMP* Ampicillin; *R* Resistant; *S* Susceptible
[a,d] The Strains of *S. aureus* NCTC 8325 and *S.aureus* MW2 (pulsed-field type USA400) were used as references in the pulsed-field gel electrophoresis
[b] The Strain CCM 3953 (ATCC 25923) was used as an international standard reference for quality control in antibacterial disk susceptibility testing
[c,e] The international strains of *S. aureus* FRI 137 and *S. aureus* FRI 361were used as references isolated from food samples

Antimicrobial susceptibility testing

Strains were tested for susceptibility to a panel of six antibiotics on Mueller-Hinton agar (HiMedia Laboratories, India) using breakpoints published by the Clinical Laboratory Standards Institute (CLSI 2010). The antibiotics were as follows: penicillin, tetracycline, erythromycin, oxacillin, cefoxitin, and ampicillin. Reference strains *S. aureus* CCM 3953 (ATCC 25923) and *E. faecalis* CCM4224 served as quality control. The inoculated plates with antibiotics were incubated for 18–24 h at 37 °C. Additionally, MRSASelect chromogenic media from Bio-Rad (USA) was used to confirm methicillin-resistant strains.

PFGE analysis

PFGE analysis of chromosomal DNA from *S. aureus* strains was performed with the restriction enzyme *Sma*I. The procedure was performed as described by Peles et al. (2007).

Briefly, bacterial suspensions were mixed with 2 % SeaKem Gold agarose (Cambrex Bioscience, USA), dispersed into molds, and set at 4 °C. Plugs were transferred to 3 ml EC lysis solution with the addition of RNAse, lysosyme and lysostaphin (Sigma-Aldrich) and were incubated overnight at 37 °C. Each plug was incubated overnight at 54 °C in 3 ml of ESP buffer (ES buffer with 1 mg/ml proteinase K (Sigma-Aldrich) and washed four times in Tris-EDTA buffer.

Plugs were digested with 40 U *Sma*I at 25 °C. Separation was performed with a CHEF DR-II (Bio-Rad, USA) pulsed-field electrophoresis system. The buffer solution was 0.5× Tris–borate–EDTA (1 M Tris, 0.01 M EDTA, 1 M boric acid) and running parameters were as follows: 200 V (6 V/cm); temperature, 14 °C; initial switch, 5.3 s; final switch, 35 s; and time, 18.4 h. *Staphylococcus aureus* NCTC 8325 and MW2 were used as reference strains. Results were analysed and interpreted using Tenover's criteria (Tenover et al. 1995), and DNA restriction patterns were analysed using the software BioNumerics version 7.0 (Applied Maths, Belgium).

The cluster cutoff value was set to 88 % as the similarity coefficient to define the pulsed-field type (PFT) clusters. Isolates with 100 % similarity were assigned to the same PFGE genotypes, and those with similarities ranging from 88 % to 99 % were designated as subtypes in one cluster.

Statistical analysis

Fisher's exact test was used to assess significance between PFGE patterns within the enterotoxins groups, origins and antibiotic resistance. A significance level of $p<0.05$ and two-tailed p values were defined using online sources VassarStats: Website for Statistical Computation (Richard Lowry, USA).

Results

All strains were confirmed as *S. aureus*, due to the detection of the gene SA442 and the target 16S rRNA. This molecular identification was in concordance with phenotypic characterisation, namely Gram positive cocci present in clusters, catalase and coagulase production. Incubation on chromogenic agar revealed pink (n=33), orange (n=55) and double-color (pink-orange, n=5) colonies, consistent with *S. aureus* reference strains. *Staphylococcus aureus* strains demonstrated α-hemolysis 18.2 % (n=17), β-hemolysis 28 % (n=26), double (α–β)-hemolytic 42 % (n=39) and 11.8 % (n=11) demonstrated weak-hemolytic activity (Table 1). Ten *S. aureus* strains, isolated from milk products (n=8), confectionery product (n=1) and fish fillets (n=1) were resistant to oxacillin and cefoxitin, grew on MRSA selective medium and were

*mec*A positive. All strains did not harbor *van*A or *van*B genes. A high percentage of the isolates demonstrated resistance to penicillin (n=77; 82.8 %) and ampicillin (n=74; 79.6 %); and in 72 strains *bla*Z was detected. Moreover, susceptibility to erythromycin and tetracycline was 62.4 % and 87.1 %, respectively. In total, 7.5 % (n=7) of the strains were susceptible to all antibiotics tested (Table 1); 73 *S. aureus* isolates (78.5 %) were resistant to at least one antimicrobial, and 26 (28 %) to three or more antimicrobials (Table 1). The presence of the *bla*Z gene was statistically associated with the resistance to ampicillin and penicillin (P<0.001).

Gene coding for enterotoxins A–E and G–J was detected amongst the strain collection. The results of the multiplex PCR analysis of all strains of *S. aureus* are shown in Table 1. One or more *se* genes were carried by 82.8 % of the isolates. Thirty seven strains (39.8 %) harboured just one *se* gene and the remaining 30 isolates (32.3 %) carried more than one gene. Twenty *se* genotypes were observed, the most commonly detected combinations were *sed selj, see seg sei* and *seg sei*, with 16, 13 and 17 % respectively. The isolates collected from dairy products demonstrated a lower incidence of *se* genes. Thirty-two strains isolated from dairy products harboured the enterotoxin gene *sec*, together with at least one other *se* gene. Furthermore, strains obtained from fermented meat products showed a higher incidence and variety of enterotoxins. The most prevalent gene was *sec* (n=49), followed by *sea, seg* and *sei.* The enterotoxin gene distribution was determined as follows: 49 foodborne strains were positive for SEC, 21 strains for SEA, 18 strains for SED, 20 strains for SEG, 18 strains for SEI, and 14 strains for SEH and SEJ (P<0.001).

A total of 93 *S. aureus* strains, including five reference strains, were analysed for genetic relatedness using PFGE, with an aim to identify the possible sources of food contamination. Pulsotypes obtained by macrorestriction analysis with *Sma*I were grouped using 11–17 fragments with a range of lengths between 15 and 700 kbp and compared to each other. Analysis revealed 24 main PFGE profiles (designated by symbols A to X), and these were detected at a similarity level of 88 % (Fig. 1). Fifty-seven milk product strains of *S.aureus* were separated into 19 different PFGE profiles. Furthermore, seven isolates from meat products were separated into six PFGE clusters (D, F, I, L, P and R). Interestingly, *S. aureus* strains STA 018 and STA 029, (both from sheep's milk) demonstrating a greater than 99 % similarity in PFGE dendrogram. These strains were isolated from same district and are assigned as an E_1 PFGE pattern. The isolates of STA 024 and STA 025 isolated from raw milk from different districts were assigned as a B_1 PFGE pattern. Isolates from milk samples from Újezd u Brna were divided into four main PFGE types (A, H, J and T) and eight subtypes (A_1, A_2, H_1, H_2,H_6, H_8 and J_1). The most heterogeneous was the PFGE profile H, which contained four strains from raw cow's milk, two from confectionery products, four from meat products, one from cereal porridge, one from spinach, and these 12 strains demonstrated the presence of one or more enterotoxin genes. The majority of *S. aureus* strains harbouring *sec* and *sed* genes were grouped into their own separate pulsotype patterns. Milk-product borne strains harbouring the *sec* gene were grouped into an E pulsotype exhibiting 76 % homology. Each of PFGE profiles V, W and X consisted of only one strain and were sourced from differing food products. It was noted that all enterotoxigenic isolates were resistant to penicillin. The enterotoxin positive pulsotypes exhibited 70–92 % homology. Non-enterotoxigenic *S. aureus* foodborne strains were assigned to different PFGE profiles with the exception of strains STA 067, STA 069 being grouped into the R_1 PFGE profile with 96 % homology. PFGE results demonstrated that the strains isolated from certain types were closely related. However, the *S. aureus* strains analysed from various food samples showed a high genetic diversity, suggesting that bacterial contamination sources of these products are multiple.

Discussion

In this study, we characterised 93 *S. aureus* strains collected from 11 regions in the Czech Republic. Phenotypic and genotypic analysis was performed by determining enterotoxin gene presence, antimicrobial susceptibilities, antibiotic resistance gene carriage and PFGE profile analysis.

Out of 93 foodborne strains, 17 (18.2 %) demonstrated α-haemolysis, 26 (28 %) β-haemolysis, 39 (42 %) were double α–β haemolysis and 11 (11.8 %) demonstrated γ-haemolysis. Our results are in agreement with El-Jakee et al. (2008), who reported that 89.7 % of the *S. aureus* isolates (from different sources) were haemolytic on sheep blood agar and 10.3 % were non-haemolytic. In a north Portugal study, Pereira et al. (2009) reported on *S. aureus* strains isolated from milk, dairy, meat and other food products. The authors found 81 % of strains were β-haemolytic, 8 % were α-hemolytic, 11 % were γ-haemolytic, consistent with our study.

Food is an important factor for the transfer of antibiotic resistance genes. The antibiotic resistance genes present on mobile genetic elements of *S. aureus* can be horizontally transferred between strains, and this process plays an important role in bacterial evolution (Malachowa and DeLeo 2010). To date, many researchers have reported resistant strains of foodborne *S. aureus* from many countries (Chao et al. 2007; Pesavento et al. 2007; Pu et al. 2011). *Staphylococcus aureus* has developed penicillin resistance and multidrug resistance worldwide, although reported prevalence rates indicate that wide variations exist regionally. Depending on the origin of the food samples, the prevalence of penicillin and ampicillin resistant *S. aureus* strains ranges from less than 10 % to more than 60 % (Jørgensen et al. 2005; Anderson et al. 2006; André

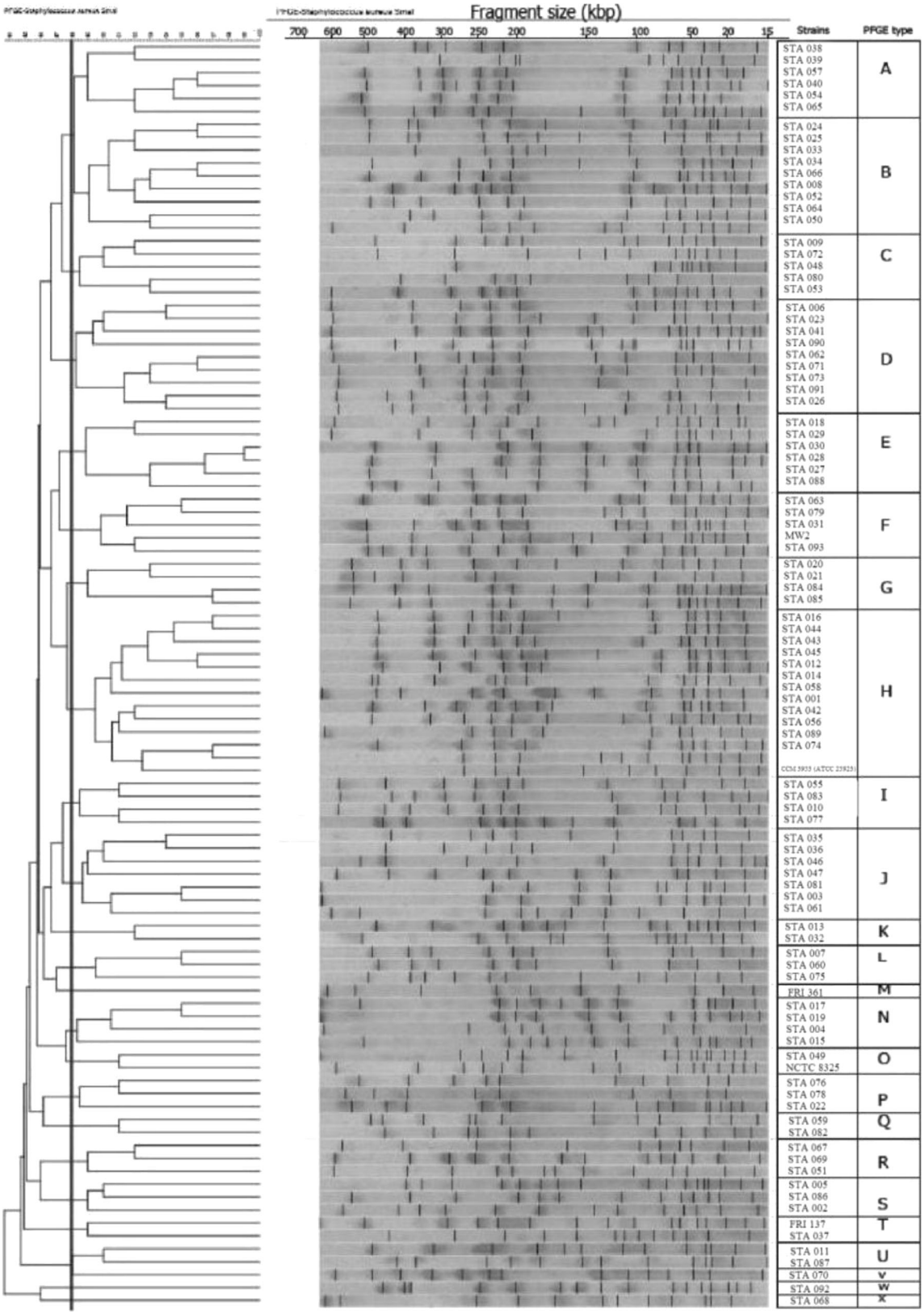

Fig. 1 Dendrogram of PFGE profile showing relationship between 93 *S. aureus* strains isolated from food samples. The cluster cutoff was set at 88 % similarity

et al. 2008). In our study, resistance to β-lactams such as penicillin and ampicillin was evident in 82.8 and 79.6 % of strains respectively; moreover, 72 strains demonstrated presence of the *blaZ* gene. Our results are in agreement with the

study performed by Peles et al. (2007). In our study, only seven (7.5 %) *S. aureus* isolates from various foods were susceptible to all tested antibiotics, while 17.2 % of *S. aureus* isolates showed multidrug resistance (resistant to at least three antimicrobials). Susceptibility to erythromycin and tetracycline was prevalent (87.1 % and 62.4 %, respectively) among our *S. aureus* strains. Comparison of our results to antimicrobial resistance rates of *S. aureus* isolates from food samples in the Czech Republic and other countries (Chao et al. 2007; Sauer et al. 2008; Aydin et al. 2011b; Pu et al. 2011) demonstrate a high degree of concordance. Of 93 *S. aureus* strains analysed, ten (10.75 %) harboured the *mecA* gene and this was predominantly from milk products (n=8). Each *mecA* positive strain demonstrated resistance to oxacillin and cefoxitin, and was able to grow on MRSAselect agar. The presence of MRSA in food products has been well documented, with the following rates reported: Italy 3.8 % (Normanno et al. 2007), Netherlands 2.5 % (van Loo et al. 2007), Spain 1.6 % (Lozano et al. 2009), Canada 6.4 % (Weese et al. 2010), and Korea 2.4 % (Rhee and Woo 2010). Normanno et al. (2007) reported that of the 160 analyzed *S. aureus* strains, six were *mec*A positive and derived from six different samples; four isolates were from bovine milk and two from dairy products (pecorino cheese and mozzarella cheese). All of our food-derived strains did not carry *vanA* and *vanB* genes. Růžičková et al. (2008) also found that all *S. aureus* strains isolated from different food samples were susceptible to vancomycin. Methicillin-resistant *S. aureus* (MRSA) causes hospital-associated and community-associated infection, and the presence of MRSA strains in various food samples has been reported (Lu et al. 2010; Pu et al. 2011). In Czech Republic, Bardoň et al. (2006) found that 78 % of *S. aureus* isolates from animal-derived food were resistant to penicillin, and 4 % to oxacilin. In one survey in the United States, 67 % of *S. aureus* isolates from retail meats were resistant to tetracycline, 30 % to erythromycin, 13 % to ciprofloxacin, and 3 % to gentamicin (Pu et al. 2011). Aydin et al. (2011b) in Turkey reported that 18.2 % of *S. aureus* isolates from retail meats exhibited erythromycin resistance, 15.6 % exhibited resistance to tetracycline, 13 % to trimethoprim/sulfamethoxazole, and 6.5 % to gentamicin. Development of multidrug resistance in *S. aureus* strains from food sources would present an enormous challenge to the treatment of *S. aureus* infections in both humans and animals.

Staphylococcus aureus produces a wide variety of exotoxins, including staphylococcal enterotoxins (SE) belonging to a family of pyrogenic toxin superantigens (SAg), and SE-contaminated foods are often involved in outbreaks. SEA, either alone or together with other SE, is considered the main cause of staphylococcal food poisoning (SFP) throughout the world, followed by SEB, SEC, SED and SEE. All staphylococcal superantigens are encoding on mobile genetic elements, such as plasmids, prophages, *S. aureus* pathogenicity islands (SAPI), genomic islands νSa, and on staphylococcal cassette chromosome (SCC) elements. A previous study (Pu et al. 2011) analysed enterotoxin genes (*sea* to *sej*) in *S. aureus* and revealed a wide diversity in the prevalence of strains harboring these toxin genes. Our study strains revealed a wide variability in the harboring of virulence genes. 82.8 % of our strains were carrying one or more of the toxin genes tested, consistent with 84.9 % of foodborne strains harbouring enterotoxin in a United States study (Pu et al. 2011). In Portugal, Pereira et al. (2009) observed that 68.2 % of 148 foodborne *S. aureus* isolates from various foods harboured genes coding for one or more enterotoxins. In another study, Normanno et al. (2007) found a similar prevalence in Italy, in which 59.8 % of the *S. aureus* strains isolated from milk, dairy and meat products produced enterotoxins. We can use information about enterotoxins to determine host–pathogen relationships, and this may provide information to help trace probable sources of contamination. Enterotoxigenic *S. aureus* strains may harbor several of these genes due to the varying modalities of gene transfer; e.g., SE genes can be located on plasmids (*sed* and *sej*), phages (*sea* and *see*), pathogenicity islands (*seb* and *sec*) and chromosomes (*seg, seh* and *sei*). In our study, *sec, sea, sed, seg* and *sei* were the most commonly detected genes, with 49 strains with SEC, 21 strains with SEA, 18 strains with SED, 20 strains with SEG, 18 strains with SEI and 14 strains with SEH and SEJ.

In this study, *sec* was detected in 52.6 % of enterotoxigenic *S. aureus* strains, and these findings are similar to those reported by Jørgensen et al. (2005), who demonstrated that 37.2 % (96 isolates) of *S. aureus* strains contained *sec* gene. The *seg* and *sei* genes encode enterotoxin gene cluster (egc), and they are usually detected together (Jarraud et al. 2001). However, in this study, *seg* and *sei* genes were detected singly in a nine strains (8.7 %). The *sed* and *sej* genes are found on the plasmid pIB485, and subsequently they are usually found together (Zhang et al. 1998). In our study, two (2.2 %) of *S. aureus* isolates contained *sed* and no isolates contained *sej*. Srinivasan et al. (2006) reported that 52.6 % of *S. aureus* strains from cow's milk contained *sed*, but none contained *sej*.

These results suggest that these genes maybe found either alone or together on mobile elements such as plasmids and genomic islands in the same strain (Srinivasan et al. 2006). Enterotoxin SEH has been demonstrated to elicit emetic activity, a causative agent for food poisoning; it is encoded on the chromosome. In this study, *seh* was detected as the only enterotoxin gene in one strain, and totally in five (5.4 %) of the isolates. *seh* and *seh* combinations with other enterotoxin genes were detected, most often *sea, seb*, and *sec*, which is in agreement with that reported by Aydin et al. (2011a). Among all *S. aureus* strains, 53.9 % were positive for the classical SE genes (SEA through SEE); these enterotoxins can cause staphylococcal food-poisoning.

In this study, the *S. aureus* strains produced various PFGE patterns, and these were not necessarily associated with different food samples. This may be a result of multiple sources of *S. aureus* contamination, including from production and processing environments, equipment, and personnel. PFGE patterns were shown to correlate with the presence of specific toxin gene profiles and antibiotic resistance profiles, which is in agreement with Aydin et al. (2011a). Also, Fueyo et al. (2005) reported that certain correlations exist between these factors. Our PFGE pattern analysis did not show genetic-relatedness of enterotoxigenic and non-enterotoxigenic *S. aureus* isolated from foods. PFGE is not an ideal typing method for determination of any genetic relationships between enterotoxigenic and non-enterotoxigenic strains. Non-enterotoxigenic *S. aureus* food isolates collected different PFGE profiles, but sometimes housed the same PFGE profile with enterotoxigenic strains. These findings agree with those of Jørgensen et al. (2005), who genotyped 306 Norwegian *S. aureus* isolates by PFGE and found that non-enterotoxigenic strains belonged to ten different PFGE clusters. Moreover, researcher didn't find any genetic relationships between PFGE profiles of enterotoxigenic and non-enterotoxigenic strains, using PFGE after digestion of the DNA with *Sma*I endonuclease. However, some researchers have reported that PFGE patterns do not correlate with the presence of specific toxin gene profiles (Srinivasan et al. 2006; Wang et al. 2012). In the present study, common PFGE pulsotypes were shared with *S. aureus*. Our results showed that genomic DNAs from all of our test strains (non-ST398) were able to be digested with *Sma*I. The DNA of *Sma*I-nontypeable ST398 isolates was partially resistant to *Xma*I, but was able to be digested with *Cfr9*I.

The combination of genotypic and phenotypic methods may be used to trace the contamination of the *S. aureus* strains due numerous sources, pre-processing environments, processing areas and the market place. The virulence pattern obtained from food isolates showed that the contamination with *S. aureus* maybe of animal origin or of human biotype.

Conclusions

Staphylococcus aureus is a major contributor to microbial contamination of food products. In the present study, nearly 75 % of the *S. aureus* strains demonstrated penicillin and ampicillin resistance; this data suggests that foodborne strains may present an important source of resistance to the treatment of human and animal infections. The high prevalence of enterotoxin genes is evident in foodborne isolates, and further studies into the prevalence of enterotoxin genes may contribute to a better understanding the role of *S. aureus* in food poisoning cases and outbreaks. Our results demonstrate the high prevalence of toxin genes in foodborne *S. aureus*; all these strains provide potential risk for causing food poisoning. PFGE analysis showed a high genetic relatedness among strains. Further research is needed to determine PFGE relatedness of enterotoxigenic and non-enterotoxigenic *S. aureus* isolates from foods.

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