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OCCURRENCE OF ENTEROTOXIGENIC *STAPHYLOCOCCUS AUREUS* IN FOOD

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Abstract: Gastroenteritis is one of the most frequent microbial diseases, which is caused by the ingestion of food contaminated with staphylococcal enterotoxins. In our study, the production of staphylococcal enterotoxins A, B (SEA, SEB) and the presence of respective staphylococcal enterotoxin genes were investigated in the field *S. aureus* isolates obtained from foods and food industry manufactures in East Slovakia. Radioimmunoassay (RIA), polymerase chain reaction (PCR) and dot-blot hybridisation were used for examination. The ability to synthetise enterotoxins was found in 20 (39.2%) of the total number of 51 isolates. Production of SEA was recorded in 3 (5.9%), production of SEB in 12 (23.5%) and production SEA together with SEB in 5 (9.8%) staphylococcal isolates. Nine (47.4%) sheep cheese isolates of the total number of 19 produced enterotoxins, especially SEB (36.8%). *S. aureus* isolates from pasta were enterotoxigenic in 6 cases (33.3%). The synthesis of enterotoxins was not detected in Bryndza cheese and sausages isolates. One enterotoxigenic isolate was obtained from smears of technological equipment and 4 isolates from throat and nasal swabs. No differences in results were recorded between RIA and PCR as well as PCR and dot-blot hybridisation. Our results suggest that it is of special importance to follow the presence of enterotoxigenic *S. aureus* strains in foodstuffs, especially for protecting the consumers from food poisoning.

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INTRODUCTION

One of the most frequent foodborne microbial diseases is staphylococcal food poisoning (SFP) which is caused by *S. aureus* metabolites. Of the many extracellular toxins which are thought to contribute to the pathogenicity of *S. aureus*, staphylococcal enterotoxins (SEs) pose the greatest risk to consumer health. Staphylococcal enterotoxins are low molecular weight proteins (MW 26,900-29,600), which are usualy divided into 7 serotypes: SEA, SEB, SEC1-3, SED, SEE [24]. The synthesis of other enterotoxins was also found in *S. aureus:* SEG, SEH, SEI, SEJ, SEK [14].

Heat resistance is one of the most important physical and chemical properties of SEs, which means that biological activity of toxins remains unchanged even after thermal processing of food. After ingestion of contaminated food, toxins are resorbed into the blood in the gastrointestinal tract, activate an emetic reflex, cause nausea, emesis, abdominal cramps and diarrhoea [25].

Genetic variation among *S. aureus* strains has been shown to be associated with pathogenic potential. Staphylococcal enterotoxin genes (*ses*) are characterised by a great percentage of nucleotide sequence identity [3]. They are carried on plasmids, the family of staphylococcal

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Table 1. Total number of enterotoxigenic *S. aureus* isolates in samples of food processing manufactures.

	Foods		Food processing manufactures	Total	
	n	%	n	n	%
Total number of *S. aureus* isolates	43		8	51	
Number of enterotoxigenic *S. aureus*	15	34.9	5	20	39.2
Genotypes					
sea$^+$*seb*$^-$	3	7.0	-	3	5.9
sea$^-$*seb*$^+$	8	18.6	4	12	23.5
sea$^+$*seb*$^+$	4	9.3	1	5	9.8
sea$^-$*seb*$^-$	28	65.1	3	31	60.8

Table 2. Number of enterotoxigenic *S. aureus* in food samples.

	Sheep cheese		Bryndza cheese		Sausages		Pasta	
	n	%	n	%	n	%	n	%
Total number of *S. aureus* isolates	19		2	2			18	
Number of enterotoxigenic *S. aureus*	9	47.4	-	-			6	33.3
Genotypes								
sea$^+$*seb*$^-$	1	5.3	-	-	-	-	2	
sea$^-$*seb*$^+$	7	36.8	-	-	-	-	1	
sea$^+$*seb*$^+$	1	5.3	-	-	-	-	3	
sea$^-$*seb*$^-$	10	52.6	2	100	2	100	12	66.7

bacteriophages and mobile genetic elements, such as recently described staphylococcal pathogenicity islands (SaPIs) [12, 14, 20], which transfer horizontally between strains.

Only limited data have been presented about the occurrence of enterotoxigenic *S. aureus* strains in foods in Slovakia [6]. Therefore the aim of our study was to investigate the production of staphylococcal enterotoxins A, B (SEA, SEB) and the presence of respective genes in the field *S. aureus* isolates obtained from various kinds of foods, and from food industry manufacturers in some Slovak regions.

MATERIALS AND METHODS

Reference *S. aureus* strains. The reference strains used (positive controls) were *S. aureus* FRI 722-SEA (Food Research Institute, University of Wisconsin, USA) and *S. aureus* CCM 5757-SEB (Czechoslovak Collection of Microorganisms, Brno, Czech Republic). As negative controls, nonenterotoxigenic strains of *S. aureus* CCM 2351 (α-hemolysin) and *S. aureus* CCM 6188 (β-hemolysin) were used (Czechoslovak Collection of Microorganisms, Brno, Czech Republic).

Isolates of *S. aureus.* Fifty one field *S. aureus* isolates were obtained from food samples (sheep cheese, Bryndza cheese, pasta, sausages) and from food manufacturers (smears of technological equipment, throat and nasal swabs from food handlers). The microbiological examination of samples was based on STN-560089 (ISO 6888) [23]. The working cultures of isolates were prepared in BHI (Brain Heart Infusion) broth at 37°C for 18 hours and tested by RIA.

Radioimmunoassay (RIA). Tracers of I^{125}-SEA and I^{125}-SEB were prepared by the chloramine T method [7]. Radioimmunoassay was performed according to Gondol' *et al.* [8].

Isolation of DNA. Total genomic DNA of *S. aureus* was isolated by phenol-chloroform method [21]. Lysates of colonies were prepared according to McLauchlin *et al.* [11].

Polymerase Chain Reaction (PCR). Johnson *et al.* [10] described oligonucleotide primers used. $MgCl_2$ (3.0 mM), AmpliTaq polymerase (2.0 U) (Perkin Elmer), nucleotide mixture (dNTPs) (0.2 mM) and primers (0.3 μM) were added into the PCR reaction buffer (10 mM TRIS-HCl pH 8.3, 50 mM KCl, 1% gelatine) (Perkin Elmer). Thermocycler Genius (Techne) was used to perform PCR which comprised of initial denaturation (94ºC, 120 s) followed by 35 cycles of denaturation (94ºC for 60 s), annealing (55ºC for 30 s) and extension (72ºC for 30 s), with a final extension cycle of 150 s at 72ºC. The resulting amplicons were detected on 2% agarose gel after ethidium bromide staining. The size of fragments was 120 bp for *sea* and 476 bp for *seb*.

Preparation of DNA probe and dot-blot hybridisation. DNA probe for detection of *S. aureus sea* gene was selected on the base of previously described nucleotide sequence of gene [2], and prepared by amplification of the target sequence according to Johnson *et al.* [10] internal to the coding region for *sea* gene. The probe was labelled by means of Dig DNA Labelling and Detection Kit (Boehringer Mannheim, Germany). DNA of *S. aureus* isolates was denatured (95ºC, 10 min), transferred onto Hybond-N nylon membrane (Amersham), fixed at 80ºC for 2 hours and hybridised with labelled probe (18 hours, 68ºC). Samples were immunochemically detected using the manufacturer's instructions.

RESULTS

The production of enterotoxins was found in 20 (39.2%) of a total number of 51 *S. aureus* isolates obtained from food samples and food-processing manufacturers. The

synthesis of SEA was recorded in 3 (5.9%), SEB in 12 (23.5%), both SEA and SEB in 5 (9.8%) of staphylococcal isolates (Tab. 1). The largest rate of enterotoxigenic *S. aureus* was found in sheep cheese (47.4%), with prevalence of SEB (36.8%) (Tab. 2). In the case of 18 isolates of *S. aureus* from pasta, 6 (33.3%) were found to be enterotoxigenic. Neither synthesis of SEA nor SEB were proved in Bryndza cheese and sausages isolates. One enterotoxigenic isolate was obtained from the technological equipment smears, 4 isolates from the throat swabs of food handlers. A comparison of the results for SEA and SEB production *in vitro* (as detected by RIA) and results of amplification of the respective toxin gene fragments by PCR was made. No differences in results were recorded between RIA and PCR.

Dot-blot hybridisation was also used for examination of all isolates to detect *sea* gene. Hybridisation signals were observed in the case of *S. aureus* in which the presence of *sea* gene and the production of SEA were detected previously by PCR and RIA (SEA^+SEB^-, SEA^+SEB^+). Reaction with probe was not obtained in isolates with production of SEB (SEA^-SEB^+) or in nonenterotoxigenic isolates (SEA^-SEB^-). An agreement between results of PCR and dot-blot hybridisation was observed.

DISCUSSION

The literature shows very variable results concerning the occurrence of enterotoxigenic S. *aureus* strains in foods. This is probably due to the differences among the kinds of examined foods, number of samples, in detection methods used, and in the ecological origin of strains.

The rate of enterotoxigenic *S. aureus* isolates from the total number of 51 isolates obtained from our samples was 39.2%. A lower occurence of enterotoxigenic strains (36.4%) was found by Tsen *et al.* [26] when examined *S. aureus* strains from Chinese sausages, frozen and other foodstuffs, as well as by Rosec *et al.* [19] (30.5%) in a study of *S. aureus* from various foods (cooked meals, meat, pasta, cheeses). In contrast, De Buyser *et al.* [4] determined production of SEs by routine analysis of foods only in 24% of *S. aureus* strains, while from cases of staphylococcal food poisoning - in 80% strains.

In comparison with literature data which report that SEA is mostly involved in outbreaks of staphylococcal food poisoning [15, 16, 29], the largest percentage of SEB producing *S. aureus* isolates (23.5%) was found in our study. Similar results were reported by Ng and Tay [13] after examination of samples of local drinks and food contaminated with *S. aureus* and by Udo *et al.* [27] in staphylococci isolated from the nasal and hand swabs of restaurant workers.

Contamination of food products with *S. aureus* pathogens may result from their presence in the basic raw material - milk [1, 18]. This is of great importance, especially in countries with large production of dairy products such as cheeses [28]. In Slovakia, sheep cheese and Bryndza cheese are considered to be traditional products, which are mostly made from unpasteurized milk [22] and therefore can contribute to the sources of staphylococcal enterotoxicosis. In our study, the production of enterotoxins was observed in nearly 50% (47.4%) of *S. aureus* sheep cheese isolates, with prevalence of SEB (36.8%). Similar results were presented by Fotta *et al.* [6] who detected enterotoxin production in 54.2% of *S. aureus* strains from sheep lumpy cheese (SEB 26.9%). In contrast, Grieger [9] determined in sheep cheese the ability to synthetise enterotoxins only in 3 (8.8%) of 34 collected *S. aureus* strains. Pasta products are also supposed to be the source of enterotoxigenic strains, which has been confirmed by Rosec *et al.* [19] and Fotta *et al.* [6].

The production of the same enterotoxins was observed in staphylococcal isolates collected from foods, as well as from nasal-throat swabs of food handlers and smears of the technological equipment from food-processing manufacturers. The above-mentioned results confirm the fact that man is the main staphylococcal reservoir and vector which is of special importance for food contamination.

By immunochemical methods, such as RIA, both the production of enterotoxins by strains and the presence of toxins in food are determined. On the other hand, the molecular-genetic methods are able to detect the potential of strains to produce SEs, especially in cases when toxin genes are not expressed due to various reasons. Detection of *S. aureus* strains which harbour the gene for SEA synthesis is important because the SEA is toxic in low concentrations (0.6 ng/ml) [5]. Therefore, we attempted to involve not only PCR but also dot-blot hybridisation into the detection of *sea.* The results of dot-blot hybridisation were consistent with those of PCR and RIA. However, as compared with PCR (detection limit 1 μg/ml), the lower detection limit of dot-blot was achieved (1 ng/ml), which corresponds with the data of Zschöck *et al.* [29]. Rifai *et al.* [17] recommended the dot-blot hybridisation for a precise study of previously isolated staphylococcal strains, but not for the direct determination of toxigenic microorganisms from samples.

CONCLUSIONS

As shown by our results, the enterotoxigenic *S. aureus* strains have occurred in foods and food-processing manufacturers in eastern Slovakia. For this reason, the estimation of SEs production is necessary to protect the health of consumers.

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