

The enterotoxigenic potential of *Staphylococcus aureus* bacteria isolated from various types of retail meat

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Abstract

Staphylococcus aureus produces a variety of toxins, some of which may cause staphylococcal intoxication. The aim of this study was to isolate *S. aureus* bacteria from samples of raw meat from the retail market in the Czech Republic and to determine the prevalence of genes encoding the production of staphylococcal enterotoxins in them. On the basis of examination of 503 samples of pork meat and pork liver, chicken, beef and venison, we found that the profiles of the genes encoding enterotoxin production differ in isolates from individual types of retail meat. In terms of public health, the greatest risk is seen in pork meat and pork liver in which strains of *S. aureus* capable of producing enterotoxin H, which may cause staphylococcal enterotoxigenicosis in consumers, were frequently detected.

Beef meat, chicken meat, pork meat, retail market, venison

Introduction

Staphylococcus aureus (*S. aureus*) bacteria are a significant agent of alimentary intoxication of bacterial origin (Normanno et al. 2007). Such intoxication is caused by thermo-resistant enterotoxins that cannot be degraded even by proteases such as trypsin, renin and papain. Staphylococcal enterotoxins (SEs) are created as metabolic products in the food in which these bacteria reproduce. *S. aureus* concentrations of 10⁵ CFU per gram or ml of food are considered critical for the possible accumulation of enterotoxin with the potential to cause illness in the consumer (Lancette and Tatini 1992).

Five classical and 16 “new” staphylococcal enterotoxins have been described. The potential to cause alimentary intoxication has, however, been confirmed to date only in the “classical” enterotoxins SEA, SEB, SEC, SED and SEE and in one newly described enterotoxin (SEH) on the basis of clinical forms of illness (Schelin et al. 2011). Its ability to cause enterotoxigenicosis has been confirmed by various studies (Pospíšilová and Karpíšková 2005). Enterotoxin H is, however, difficult to demonstrate in food. The immunochemical methods usually used to determine staphylococcal enterotoxins in foods, such as ELISA (Enzyme-Linked ImmunoSorbent Assay) and ELFA (Enzyme Linked Fluorescent Assay, VIDAS), are designed merely to detect classical enterotoxins and give negative results in the presence of enterotoxin H. No information on the occurrence of strains of *S. aureus* capable of producing enterotoxin H in meat on the retail network is currently available.

The food commodities associated with the occurrence of staphylococcal enterotoxigenicosis include meat and meat products (Lancette and Tatini 1992). Humans, animals and the environment of processing and production plants may all be sources of the contamination of foodstuffs with *S. aureus* bacteria. In man, *S. aureus* bacteria may colonise the mucous membranes and skin over the long term (around 20% of adults), while around 60% of people are colonised intermittently during the course of their lives (Van Belkum et al. 2009). Livestock animals and subsequently raw materials destined for further processing

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and consumption may also be infected or colonised (Lee 2003). *S. aureus* bacteria contaminating foods and foodstuffs may come from non-heat-treated ingredients and from equipment and the environment in the food industry (Varnam and Evans 1991). The improper handling of foodstuffs in the retail network, such as their slicing and packaging, may also be another possible source of contamination (Karpíšková and Gelbíčová 2009). Proper hygiene practice and the observation of the principles of personal hygiene during food handling are important factors in limiting the occurrence of these undesirable and potentially toxinogenic microorganisms.

Materials and Methods

A total of 503 samples of meat and liver from the retail network in the Czech Republic were tested in the years 2012 to 2014. The set of samples was comprised of pork meat and liver, chicken meat, beef meat and venison. After purchase on the retail network, the samples were transported to the laboratory chilled. Swabs were taken from the surface of the meat in the laboratory with sterile sponges (3M™ Sponge-stick, USA), which were placed in bags with 30 ml buffered peptone water (BPW, Oxoid, UK) and homogenised in a device of the Stomacher type. *S. aureus* bacteria were detected in the samples by a modified method based on CSN EN ISO 6888 representing isolation on a Baird-Parker medium (Oxoid, UK) following prior enrichment in BPW incubated at 37 °C for 18 – 24 hours.

Typical colonies were seeded on a medium with the addition of 5% sheep blood (LabMediaServis, Czech Republic) and stored for subsequent characterisation. Suspect colonies were confirmed by the polymerase chain reaction method (PCR) during which a fragment specific for *S. aureus* of a size of 108 bp was detected (Martineau et al. 1998). The determination of the presence of the genes *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei* and *sej* was performed for each strain of *S. aureus* by the PCR method according to the protocol described by Monday and Bohach (1999) and Løvseth et al. (2004).

Results and Discussion

The presence of *S. aureus* was confirmed by the PCR method in 127 out of 503 samples of raw meat and liver from the retail market in the Czech Republic. The presence of one of the genes encoding the production of the monitored enterotoxins was confirmed by the PCR method in 80 isolates (62.9%). The occurrence of *S. aureus* bacteria and their toxinogenic potential by individual types of meat is shown in Plate V, Fig. 1.

S. aureus bacteria and genes encoding the formation of the monitored staphylococcal enterotoxins (*SEs*) occurred most frequently in the samples of chicken meat (42/36%). The results of detection of *SE* genes in individual types of meat are shown in Table 1. The combination of most frequently detected genes in chicken meat was *seg* and *sei*. In view of the fact that these genes have not to date been unambiguously identified as agents of staphylococcal intoxication, they do not represent a significant risk from the viewpoint of a public health safety. In the contrast, findings of strains with the potential enterotoxin H production, which did not occur in chicken meat but which were frequently detected in samples of pork meat and liver, represent a significant risk. Our results do not, however, correspond to the studies by Cha et al. (2006) and Kéroutanton (2007), according to whom strains of *S. aureus* carrying the *seh* gene occur predominantly in combination with genes encoding the formation of classical enterotoxins, largely enterotoxin A. The occurrence of strains of *S. aureus* with production of enterotoxin A is also described as the most frequent in the work by the Korean authors Cho et al. (2012), who state their finding in as many as 36.5% of strains isolated from poultry and pork meat. Detection of strains of *S. aureus* with production of enterotoxin H was not, however, performed in the given study. Only one strain of *S. aureus*, though without the formation of toxins, was detected in samples of venison. The results obtained indicate that there are differences in the level of contamination in various types of meat. Meat processing at the slaughterhouse carries greater risk from the viewpoint of occurrence of *S. aureus* than the processing of venison. In view of the low number of samples of venison analysed, however, we were not able to confirm this hypothesis by statistical analysis.

Table 1. The overview of *S. aureus* strains by commodity and genes encoding SE production

| SE gene | Pork meat and liver | Beef meat | Chicken meat |
|--------------------|---------------------|-----------|--------------|
| <i>seg sei</i> | 5 | 3 | 32 |
| <i>sec seg sei</i> | 1 | 0 | 2 |
| <i>sea seh</i> | 1 | 0 | 0 |
| <i>seb seh</i> | 1 | 0 | 0 |
| <i>see seh</i> | 1 | 0 | 0 |
| <i>seh</i> | 25 | 0 | 0 |
| <i>sea</i> | 2 | 0 | 0 |
| <i>seb</i> | 0 | 1 | 0 |
| <i>sec</i> | 1 | 1 | 0 |
| <i>seg</i> | 1 | 0 | 0 |
| <i>sei</i> | 1 | 0 | 1 |
| <i>sej</i> | 0 | 0 | 1 |

Conclusions

The occurrence of *S. aureus* strains and their toxinogenic potential was seen to differ in various kinds of meat. Pork meat and liver, in which strains of *S. aureus* with potential enterotoxin H production were detected, represent a greater risk from the viewpoint of food safety.

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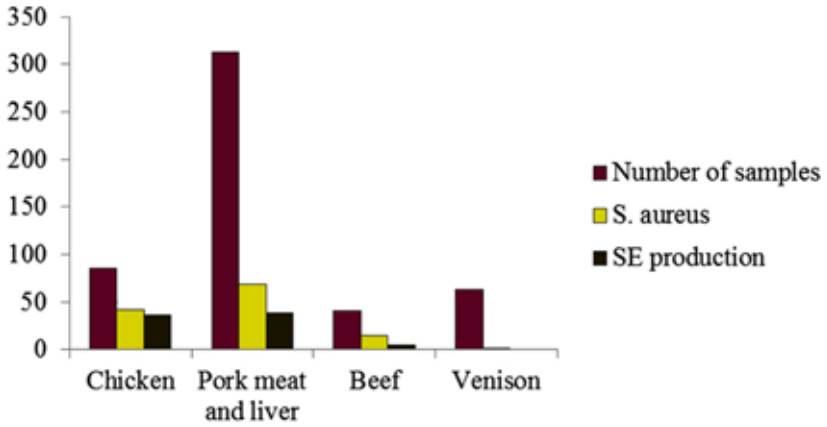


Fig. 1. Number of tested samples, samples positive for the presence of *S. aureus*, the ability to produce staphylococcal enterotoxins