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An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk

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Abstract

Mashed potato made with raw bovine milk was suspected to have been the source of a food poisoning outbreak. Almost 8×10^8 *Staphylococcus aureus* CFU g^{-1} were detected in the mashed potato. *S. aureus* was also found in bulk milk from the farm that had supplied milk for the mashed potato. Isolates from mashed potato and bulk milk were positive for the gene encoding staphylococcal enterotoxin H (*seh*), and the corresponding protein toxin, SEH, was detected by ELISA in the mashed potato. Production of SEH by *S. aureus* isolates from mashed potato ($n = 4$) and bulk milk ($n = 4$) was also demonstrated by ELISA. Sequencing of *seh* from one mashed potato isolate and one bulk milk isolate confirmed that the gene was a variant *seh*, and that the genes in both isolates were identical. Macrorestriction of chromosomal DNA with *Sma*1 followed by pulsed-field gel electrophoresis of *seh*-positive *S. aureus* from mashed potato and bulk milk revealed indistinguishable banding patterns between isolates from both sources. It seems likely that raw bovine milk used in the preparation of mashed potato contained *S. aureus* that subsequently produced sufficient SEH in the mashed potato to cause food poisoning.

Keywords: Staphylococcal food poisoning; Staphylococcal enterotoxin; SEH; Raw milk

1. Introduction

Staphylococcal food poisoning (SFP) is caused by ingestion of food containing preformed staphylococcal enterotoxin (SE), produced by some strains of *Staphylococcus aureus* and occasionally by other staphylococci [1,2]. Symptoms have a rapid onset (1–6 h), and typically include vomiting, diarrhoea and stomach cramps [1]. An outbreak of SFP is usually suspected if more than one person is affected with these symptoms shortly after eating. However, food poisoning caused by the emetic toxin of *Bacillus cereus* is an important differential to SFP because it causes similar symptoms also with a rapid onset [3].

To date, 18 SEs have been described and designated SEA–SEE, SEG–SER and SEU [4–11]. Some of the SEs lack the ability to cause emesis (SEL, SEQ) [11,12], and others have yet to be tested for emetic potential (SEJ, SEK, SEM–SEP, SER and SEU). These SEs are more appropriately referred to as SE-like proteins (SEl) [13], and the role of the SEls in SFP is unclear. Remaining SEs have been implicated in outbreaks of SFP in various reports [14–19].

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Because SEs are stable with respect to heat and storage, they may be present in foods where viable *S. aureus* are absent [1]. Moreover, not all strains of *S. aureus* produce SE. Therefore, a conclusive diagnosis of SFP should be based on the demonstration of SE in the food. To date, commercial kits for identification of SEs are only available for SEA–SEE, and if none of these are present in suspected foods the identification of SE-genes in available *S. aureus* isolates by PCR may be useful.

In December 2003, five children aged 2–6 years, and three adults became sick after eating lunch together in a kindergarten. The symptoms occurred approximately 1 h after eating and included vomiting, diarrhoea and abdominal cramping. All eight persons were fully recovered 24 h after onset of disease. For the lunch, sausage and mashed potato, leftovers from a Christmas party held the night before, had been re-heated. The mashed potato had been prepared with raw milk. Not all the affected persons had eaten sausage, but all had eaten mashed potato. Based on the history, SFP from the mashed potato was suspected. The aim of the present study was to identify the cause of the food poisoning.

2. Materials and methods

Preliminary bacteriological analyses of mashed potato and sausage were performed at Labpartner, Elverum, Norway. Bacteriological analysis of bulk milk, detection of SEA–SEE and SE-genes, genotyping, and sequencing were performed at the National Veterinary Institute in Oslo (NVI). Detection of SEH was performed at the Department of Veterinary Microbiology, Faculty of Agriculture, Iwate University in Japan.

2.1. Samples

Samples of sausage and mashed potato (scraps from the lunch) were collected, chilled and submitted to Labpartner IKS for bacteriological analyses. A sample of bulk milk from the dairy farm that had supplied raw bovine milk for the mashed potato was collected in a sterile plastic vial, and sent chilled overnight to NVI.

2.2. Bacteriological analyses

Mashed potato was streaked on glass slides, air-dried and analysed by direct microscopy using Gram staining (Difco, Sparks, MD, USA). Serial 10-fold dilutions of mashed potato and sausage were prepared in peptone water and analysed by standard methods for *S. aureus* [20], using sheep blood agar (BA) (Oxoid, Basingstoke, UK) and Baird Parker agar with a rabbit plasma fibrinogen supplement (BP + RPF) (bioMérieux, Marcy-l'Etoile, France), and for *B. cereus* [21], using *B. cereus* agar (Oxoid) and BA. Bulk milk was analysed for *S. aureus* as described above, but using BA with bovine blood. Fourteen and nine *S. aureus* isolates from mashed potato and raw milk, respectively, and one isolate of *B. cereus* from mashed potato, were stored in Heart Infusion Broth (Difco) with 15% glycerine at −80 °C until further analyses.

2.3. Analyses for SEA–SEE in food samples

Mashed potato, sausage and bulk milk were tested for SEA–SEE by the Transia plate-staphylococcal enterotoxin kit (Diffchamb, Västra Frölunda, Sweden) according to the manufacturer's recommendations. The method is based on an ELISA performed on the resulting supernatant after homogenisation of 25 g of a food sample with distilled water, adjustment of pH depending on sample-type, and centrifugation. Supernatants from samples of milk and milk products are, according to the protocol, additionally subjected to dialysis against polyethylene glycol (PEG) before the ELISA. The mashed potato sample was subjected to this dialysis step because it was prepared with milk.

2.4. Analysis of toxin production by bacterial isolates

S. aureus isolates from mashed potato ($n = 14$) and from bulk milk ($n = 9$) were tested for production of SEA–SED by SET-RPLA (Oxoid). Before testing, isolates were plated on BA and incubated aerobically at 37 °C for 24 h. One colony was picked from the BA plate and inoculated into Tryptic Soy Broth (Difco) and incubated aerobically at 37 °C for 24 h. The SET-RPLA kit was then used according to the manufacturer's instructions.

Detection of *B. cereus* toxins was performed at the reference laboratory for Gram-positive spore-forming food pathogens at the Norwegian School of Veterinary Science in Oslo. One *B. cereus* isolate from mashed potato was tested by a Vero cell cytotoxicity assay [22] and a sperm motility test [23] for *B. cereus* enterotoxin and emetic toxin, respectively.

2.5. Detection of SE-genes in S. aureus isolates

S. aureus from mashed potato ($n = 14$) and from bulk milk ($n = 9$) were tested for the presence of SE-genes (*sea–see*, *seg–sej)* and the toxic shock syndrome toxin (TSST-1) gene (*tst*). DNA extraction using CTAB, and multiplex PCR (m-PCR) was performed as described previously [24]. The method includes primers for 16S rRNA for control of DNA isolation, and four positive control strains (FRI 913, 3169, R5460, R5010) that together include all the genes detected by the m-PCR. MilliQ water was used as negative control.