Innovative Application of Mass Spectrometry for the Characterization of Staphylococcal Enterotoxins Involved in Food Poisoning Outbreaks

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Staphylococcal food poisoning is one of the most common food-borne diseases (12, 15, 16), resulting from ingestion of staphylococcal enterotoxins (SEs) produced in food by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mainly Staphylococcus aureus (11). The European Food Safety Authority (2) reported that SEs were involved in 4.1% of food poisoning outbreaks, but this percentage is certainly underestimated due to poor analytical performances in the detection and identification of SEs in food remnants. Most cattle carry staphylococci on their skin and mucous membranes, which can contaminate animal-derived products. S. aureus can also be transferred into food by handlers not respecting hygienic standards during manufacturing (3) or cooking (19).

To date, 23 SEs have been described: SEA to SEIV (21). All share superantigenic activity, whereas only few of them (SEA to SEI, SER, SES, and SET) have been proved to be emetic (16, 18). Until recently, SEs were hardly distinguishable from food proteins, as SEs are small (22- to 29-kDa) proteins without any physicochemical particularities. Their detection was possible only by immunoassay-based methods, such as the enzyme-linked immunosorbent assay (ELISA). Actually, qualitative or semiquantitative commercial kits are able either to detect SEA to SEE as a whole (“total” SEs) or to differentiate four outbreaks.

Four SFP outbreaks associated with coconut pearls successively occurred in the Ile-de-France area (France) during July 2006. Out of 14 exposed people, 11 experienced nausea, vomiting, abdominal cramps, and diarrhea 2 to 7 hours after consumption of meals. One food sample from each outbreak was subjected to CPS counting, and isolated strains were analyzed by biotyping, pulsed-field gel electrophoresis, and PCR targeting genes sea to sej and 23S rRNA (14). An RT-PCR for the sea, sed, and sej genes was performed to evaluate the expression of se mRNA. All culture supernatants of isolates were also tested for production of SEA to SED by a semiquantitative SE reversed passive latex agglutination (SET-RPLA) kit (Oxoid, Dardilly, France).

More than 10⁶ CFU of CPS/g was isolated from coconut pearl outbreak samples. The isolates were identified as S. aureus. They harbored a human biotype, an undistinguishable pulsed-field gel electrophoresis profile (data not shown), and a sea, sed, and sej gene pattern, and the RT-PCR proved the expression of sea, sed, and sej mRNA. SEA and SED were detected in supernatants by SET-RPLA (Table 1). These findings suggest that the same S. aureus strain was involved in the four outbreaks.

Detection of SEs in coconut pearls was performed by the European Union Community Reference Laboratory screening and confirmatory methods (1, 10). SEA and SED were detected in and quantified for all samples (Table 1).

MS detection and quantification of SEA were performed by the Protein Standard Absolute Quantification (PSAQ) method, using [¹³C₆,¹⁵N₄]-arginine- and [¹³C₆,¹⁵N₂]-lysine-labeled SEA as a standard (5). This internal standard was added to the food extract prior to immunochromatographic enrichment of SEA toxin as described in reference 6. After in-gel trypsin digestion, peptides were analyzed by nano-liquid chromatography–MS with a QToF mass spectrometer (Waters, Milford, MA). SEA quantification was drawn from the ratio of the unlabeled (endogenous SEA) and labeled (PSAQ standard) peptide signals for the two SEA-specific peptides NVT...
TABLE 1. Results obtained for food samples

<table>
<thead>
<tr>
<th>Outbreak no.</th>
<th>CPS count (10^6 CFU/g)</th>
<th>Amt of SE (ng/g) by analysis of coconut pearl samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SEA^a</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>3.8 ± 1.6</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4.7 ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>6.7 ± 0.9</td>
</tr>
</tbody>
</table>

^a The results for analyses of isolated strains were as follows: biotyping revealed the human biotype; molecular tests (PCR targeting 23S rRNA and sea to sej and RT-PCR targeting mRNA) revealed the S. aureus sea, sed, and sej genes and mRNA; and immunological tests with supernatants (SET-RPLA, a qualitative test) revealed SEA and SED positivity.

^b Determined by microbiological tests.

^c Determined by immunological tests ([n = 3] targeting SEA to SEE. Values shown are for qualitative ELISA. All values for qualitative ELISA were >0.1.

^d Determined by quantitative MS tests ([n = 3] targeting SEA. NT, not tested.

ng, which agrees with epidemiological studies involving SEA (3, 9, 11).

In conclusion, until now both official screening and confirmatory methods based on the same immunoadassay principle have suffered from severe limitations, such as the difficulty in obtaining specific antibodies for every incriminated or suspected SE. This case-analytical study has shown the very interesting and additional information which can be retrieved from the combination of PCR-based tools and quantitative MS, compared to the single use of microbiological tests and ELISA kits, which depend on tedious development of specific antibodies. Thus, it is crucial to continue developing a new analytical complementary approach including physicochemical methods using specific SE trypsic peptides. When the availability of primary sequence information for all the known SEs is taken advantage of, specific trypsic peptides (proteotypic peptides) can be easily targeted to identify and exactly quantify most of the SEs by the present MS method and to properly characterize and investigate SFP outbreaks.

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REFERENCES


