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What place for mass spectrometry to better elucidate Food poisoning outbreaks due to bacterial toxins.

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Staphylococcal food poisoning outbreaks (SFPO) are caused by the ingestion of food contaminated with staphylococcal enterotoxins (SEs) produced by strains of *Staphylococcus aureus (S.aureus)*. To date, 33 SEs are described in the literature but only 5 classical toxins (SEA to SEE) can be routinely detectable via commercially available immunoassays (EN ISO 19020). Liquid chromatography coupled to mass spectrometry (LC-HRMS) approach is highly specific and allows the analysis of a wide range of toxins comparing with immunoassays. In this work, we propose to develop a Multiplex method by LC-MS for the detection of SEs produced by *Staphylococcus aureus* strains in culture supernatant and in food matrices.

In food, detection of SEs is based on selective capture by antibodies and targeted high-resolution LC-HRMS. Briefly; samples were incubated with magnetic beads coated with toxin-specific antibodies. After toxin extraction, on-bead trypsin digestion was performed and recovered peptides were analyzed by LC-HRMS. This multiplex method was optimized for 8 staphylococcal enterotoxins (SEA to SEE and SEG, SEH and SEI) for which antibodies are available in the commerce or produced in CBRN project. In the culture supernatant contaminated by toxins produced by *S.aureus*, LC-HRMS method based on acid precipitation protocol was developed for the detection of 24 SEs. Thus, a database of 93 specific signature peptides and LC-HRMS parameters was optimized using sequences from 24 SEs genes, including their 162 variants.

Both methods LC-HRMS were tested in case of naturally contaminated samples involved in food poisoning outbreaks and detection of emerging toxins produced in culture supernatant. Results demonstrated that this method was sensitive, specific and able to detect SEs in naturally contaminated food and gave a good agreement with the official method. The LC-HRMS method showed high performance in terms of specificity, sensitivity, and accuracy when applied to 49 enterotoxin-producing strains. SE concentrations measured depended on both SE type and the coagulase-positive staphylococci (CPS) strain. This study indicates that LC-HRMS is a relevant alternative and complementary tool to ELISA methods. The advantages of LC-HRMS clearly lie in both the multiplex analysis of a large number of SEs, and the automated analysis of a high number of samples.

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