

Research project IZS VE 05/12

Development of MALDI-TOF MS based methods for botulism outbreak investigation

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Botulinum neurotoxins (BoNTs) are the most poisonous substances known and for this reason they are also considered a possible bioterrorism weapon. BoNTs are produced by some bacteria of genus *Clostridia* especially *C. botulinum*, *C. baratii*, *C. argentinense* and *C. butyricum*. These toxins are divided in seven serotypes, A to G, based on their antigenic activity. *C. botulinum* type A, B, E and F are usually cause of human illness whereas type C and D have been shown to affect animals. The reference method for toxin detection and identification, both in food and clinical samples, is the mouse bioassay. This method is sensitive, specific and is able to measure also toxin activity but it is time consuming and requires the use of many animals that represent an important ethical issue.

Mass spectrometry has become an important analytical tool for many applications in microbiology, including bacteria identification. Recently a method for toxin detection and serotype differentiation based on LC-ESI-MS/MS and MALDI-TOF MS (Endopep-MS method), coupled with antibody purification and enrichment of toxins, it has been developed and successfully applied on sera and feces for detection of A, B, E and F toxins and promising results were obtained also for toxin C and D. Furthermore, MALDI-TOF MS technology has been recently applied also for bacteria subtyping (e.g. MRSA, *C. difficile*, Legionella) and this type of approach could be useful also for epidemiological investigation on BoNT producer bacteria.

The specific objectives of the proposal are:

- to set up and validate a mass spectrometry method for detection of A, B, E and F toxins in foods;
- to set up and validate a mass spectrometry method for detection of C and D toxins in animal origin samples;
- to implement the database for mass spectrometry identification and typing of neurotoxin producer clostridia.