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ePoster Viewing
Diagnostic bacteriology – non-culture based, including molecular and MALDI-TOF

Novel serotype-specific rapid detection method for Cronobacter sakazakii

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Background: *Cronobacter sakazakii* is an ubiquitous, opportunistic, Gram-negative bacterium, belonging to the family of *Enterobacteriaceae.* In clinical microbiology this species is of utmost interest, because its occurrence in powdered infant formula (PIF) has been associated with causing life threatening infections in neonates. Severe symptoms such as necrotizing enterocolitis (NEC), sepsis and meningitis result in a mortality rate of up to 80%. Earlier publications have reported an epidemiological connection between serotypes O1-O7 and clinical outbreaks in infants. Serotypes O1 and O2 seem to be most prevalent in PIF as well as in clinical cases followed by serotypes O4 and O7. Serotype O3 could be isolated frequently from PIF but not as often from clinical cases. So far, the occurrence of *C. sakazakii* in PIF has been monitored according to an ISO method based on standardized microbiological procedures. This laborious method consists of several cultivation- and incubation-steps, requiring up to six days for a positive result. Due to the lack of serotype-specific monoclonal antibodies (mAb), published serotyping methods are based on antisera reactions or PCR, with considerable limitations concerning cross-reactivity. Here, we present novel and highly specific sandwich enzyme immunoassays (EIAs) as rapid detection and serotyping method for *C. sakazakii*.

Material/methods: For the establishment of a specific rapid detection method, mAb against *C. sakazakii* strains were developed. For this purpose an immunogen was prepared by treating *C. sakazakii* strains with polymyxin-B-sulfate and the obtained lipopolysaccharide (LPS) preparation was used for the immunization of female mice. Mice with the highest antibody titres were used for fusion experiments. Secreted mAb were characterized using EIA, immunofluorescence, immunoblot and motility assay. Their reactivity with other serotypes, *Cronobacter* species, *Enterobacteriaceae* and Gram-negative bacteria from a strain collection comprising more than 100 strains was screened by EIA.

Results: A part of the developed mAbs showed serotype-specific binding of *C. sakazakii*. The O-antigen chain of the LPS was identified as the immunogenic target. These mAbs were applied in the establishment of highly sensitive and serotype-specific sandwich EIAs, in which detection limits as low as 10^3 CFU/ml could be realized. The applicability of the established method was demonstrated: PIF-samples spiked with low bacterial cell counts could reliably and reproducibly be detected after only 15 h of enrichment.

Conclusions: This specific and highly sensitive detection method is the first successful attempt to simultaneously and rapidly detect and serotype *C. sakazakii* using an immunobased method. In contrast to conventional methods, positive results are obtained after a simple pre-incubation of 15 h. Therefore the introduced method promises to be of great benefit for public health and has the potential of being engaged to routine diagnostic.