Title: COMPARISON OF PCR COMBINED WITH FLUORESCENCE-BASED CAPILLARY ELECTROPHORESIS AND REAL TIME PCR FOR THE MULTIPLEX DETECTION OF BACTERIAL MENINGITIS PATHOGENS

Authors: Fukasawa, L.O., Gonçalves, M.G., Salgado, M.M., Higa, F.T., Sacchi, C.T.

Institution: Instituto Adolfo Lutz (Avenida Dr. Arnaldo, 355, 11º andar, CEP 01246-902, São Paulo – SP)

Abstract:

The rapid diagnosis of bacterial meningitis is essential for the implementation of appropriate antibiotic therapy in patient reducing lethality and for interventions in control of outbreaks and epidemics of meningococcal disease. Real time PCR assays (qPCR) has been widely used in clinical laboratories to diagnose various diseases because of high sensitivity and specificity and provide results more rapidly than traditional tests as culture. However, these assays presented a limitation in the number of qPCR reactions that can be complexed, usually not exceeding four targets. In this context, our group has standardized a 6-plex PCR combined with fluorescence-based capillary electrophoresis (FCE-6-plex PCR) for the simultaneous detection of six main bacteria of meningitis: *Neisseria meningitidis* (Men), *Streptococcus pneumoniae* (Spn), *Haemophilus influenzae* (Hi), *Staphylococcus aureus* (Saur), *Listeria monocytogenes* (Lm) and *Streptococcus agalactiae* (Saga). In this study, we evaluate the performance of this FCE-6-plex PCR in routine diagnosis of bacterial meningitis, comparing their results with those obtained by a multiplex qPCR assay for Men, Spn and Hi detection. We analyzed 508 clinical samples (321 CSF and 187 sera) from patients with suspected bacterial meningitis in São Paulo city. The FCE-6-plex PCR was carried out using DNA extracted from clinical samples and six pairs of specific primers for each bacterium; each forward primer was labeled with the fluorophore FAM or HEX. The separation of PCR products were performed with capillary electrophoresis on ABI 3130xl sequencer and the data were analyzed by GeneMapper software. FCE-6-plex PCR resulted in 230 positive samples for Men, 54 for Spn, 29 for Hi, 1 for Saur, and 194 were negative. Using qPCR, we obtained 222 positive samples for Men, 60 for Spn, 30 for Hi, and 196 were negative. No sample was positive for Lm and Saga. Considering qPCR as the gold standard, the sensitivity of the FCE-6-plex-PCR was 99.3% and the specificity was 98.9%. Our data demonstrated that this novel FCE-6-plex PCR exhibited an excellent specificity and sensitivity similar to that presented by qPCR with the additional advantage of detecting 3 more pathogens. Given its high throughput potential and lower costs, it may be useful for future use in screening for different pathogens contributing to the improvement of bacterial meningitis surveillance.

Key-words: bacterial meningitis, multiplex PCR, fluorescence-based capillary electrophoresis