

Diagnosis of bacterial meningitis by real-time PCR - with focus on broad-range PCR

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ABSTRACT

The PhD study was carried out at the Department of Infectious Diseases, Skejby University Hospital, Aarhus, in close collaboration with the Department of Clinical Microbiology, Skejby University Hospital, Aarhus.

Broad-range real-time polymerase chain reaction (RT-PCR) with primers targeting conserved areas of the 16S ribosomal RNA (16S rRNA) gene allows detection of virtually all pathogenic bacteria, and subsequent DNA-sequencing may give final identification. We hypothesised that such techniques were useful for the rapid diagnosis of bacterial meningitis and had the potential for improvement. The main aim of this dissertation was therefore to develop and validate rapid and automated PCR assay based on the 16S rRNA alone and in combination with a species-specific PCR. The PCR strategies were aimed at community-acquired and ventricular-drainage related bacterial meningitis.

Broad-range real-time PCR were applied to 206 cerebrospinal fluid (CSF) specimens from patients with suspected community-acquired bacterial meningitis and from 350 CSF specimens from patients with ventricular-drainage. A two-hour combined RT-PCR, which targeted both two major meningitis causing pathogens: *Neisseria meningitidis* and *Streptococcus pneumoniae* and the 16S rRNA gene, was applied to 1015 CSF specimens.

In conclusion, broad-range RT-PCR was more sensitive than broad-range conventional PCR and microscopy and was generally comparable to culture of CSF. Supplementary broad-range RT-PCR may be useful for the diagnosis of bacterial meningitis, particularly when antimicrobial therapy has been administered or when the microscopy of CSF is negative. The diagnosis of ventricular-related bacterial meningitis, in particular, may be improved with supplementary broad-range RT-PCR by detection of more episodes of ventricular-related bacterial meningitis and by a rapid exclusion or confirmation of such infections. The combined two-hour RT-PCR strategy was more sensitive than broad-range RT-PCR alone.

In perspective, the morbidity and mortality of patients with bacterial meningitis may be reduced if such PCR techniques result in more rapid targeted antibiotic therapy and the epidemiologic surveillance may also improved.