Abstract

The aim of the present work was to evaluate the usefulness of a simplified method for DNA extraction coupled to a nested-PCR protocol, based on the amplification of pneumolysin gene fragments for the diagnosis of pneumococcal pneumonia in pediatric patients with clinical and radiological evidence of bacterial infection. Bacterial DNA was extracted from sera by boiling and used without further purification in the PCR for the pneumolysin gene. None toxic reagents were used and the necessary steps to obtain the DNA were left at a minimum; furthermore, it overcomes the use of expensive commercial kits for DNA purification. The total procedure can be completed the same day of sampling and, most important, it avoids the use of sophisticated technology. Both in vitro analytical specificity and sensitivity (10 CFU/ml) of the assay were similar to those previously reported. When clinical samples were tested, the rate of positivity was shown to be 83.3% and 71% in pediatric patients with positive (group a) and negative blood cultures (group b), respectively. In group a, DNA detection was successful in samples from children without treatment or with less than 48 h of antibiotic therapy. None amplification was obtained from sera patients with viral infection or in samples from healthy controls. The application of the strategy described in this paper substantially seems to improve the diagnostic process in a determinate group: blood culture-negative children with pneumonia.

Keywords

Streptococcus pneumoniae, pneumococcal pneumonia, nested-PCR