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Quantitative detection of *Streptococcus pneumoniae* from sputum samples with real-time quantitative polymerase chain reaction for etiologic diagnosis of community-acquired pneumonia☆

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Abstract

We assessed the clinical usefulness of a real-time quantitative polymerase chain reaction (RQ-PCR) method applied on sputum samples to identify *Streptococcus pneumoniae* in 184 consecutive patients admitted to hospital with community-acquired pneumonia. Induced sputum samples were analyzed by culture and RQ-PCR. In total, 70/184 patients (38%) were diagnosed with *S. pneumoniae*. Cultures from blood and nasopharyngeal secretions were positive in 27/179 (15%) and 42/158 (27%) cases, respectively. Pneumococcal antigen was detected in 33/169 (20%) urine specimens. In sputum samples, culture was significantly positive in 19/128 (15%), whereas a significant concentration of DNA was found by RQ-PCR in 34/127 (27%) cases ($P < 0.001$). In 28/34 (82%) patients with RQ-PCR–positive sputum samples, *S. pneumoniae* was also detected with other methods. In the 34 RQ-PCR–positive sputum samples, 17 were negative by sputum culture, out of which 14 were obtained from patients treated with antibiotics prior to sampling. *S. pneumoniae* may be rapidly diagnosed by analyzing induced sputum samples by RQ-PCR and may be particularly valuable in patients in whom antibiotic therapy has been initiated.

Keywords: *Streptococcus pneumoniae*; Real-time quantitative PCR; Pneumonia

1. Introduction

Community-acquired pneumonia (CAP) is a common disease (Jokinen et al., 1993; Macfarlane et al., 1993; Woodhead et al., 1987) representing a major cause of hospital admission and mortality of infectious origin among adults, also in high-income countries (Almirall et al., 1993; Jokinen et al., 1993; Lave et al., 1996; Marrie, 1990; Marston et al., 1997). *Streptococcus pneumoniae* is the leading cause of CAP, especially among young children, elderly people, and persons with underlying chronic diseases (British Thoracic Society, 2001). *S. pneumoniae* is also the most common pathogen causing fatal CAP (Fine et al., 1996).

Many well-designed studies of patients with CAP however have succeeded in identifying the etiologic agent in only 40% to 60% of the cases (British Thoracic Society, 2001; Fang et al., 1990). More recent studies, using polymerase chain reaction (PCR) assays on specimens from lung aspirates (Ruiz-Gonzalez et al., 1999) and blood (Menendez et al., 1999), have suggested that pneumococci probably also cause a majority of CAP cases, with undefined etiology.

In the management of patients with CAP, rapid identification of the microbiologic cause is important to permit antibiotic selection directed at the causative agent. However,

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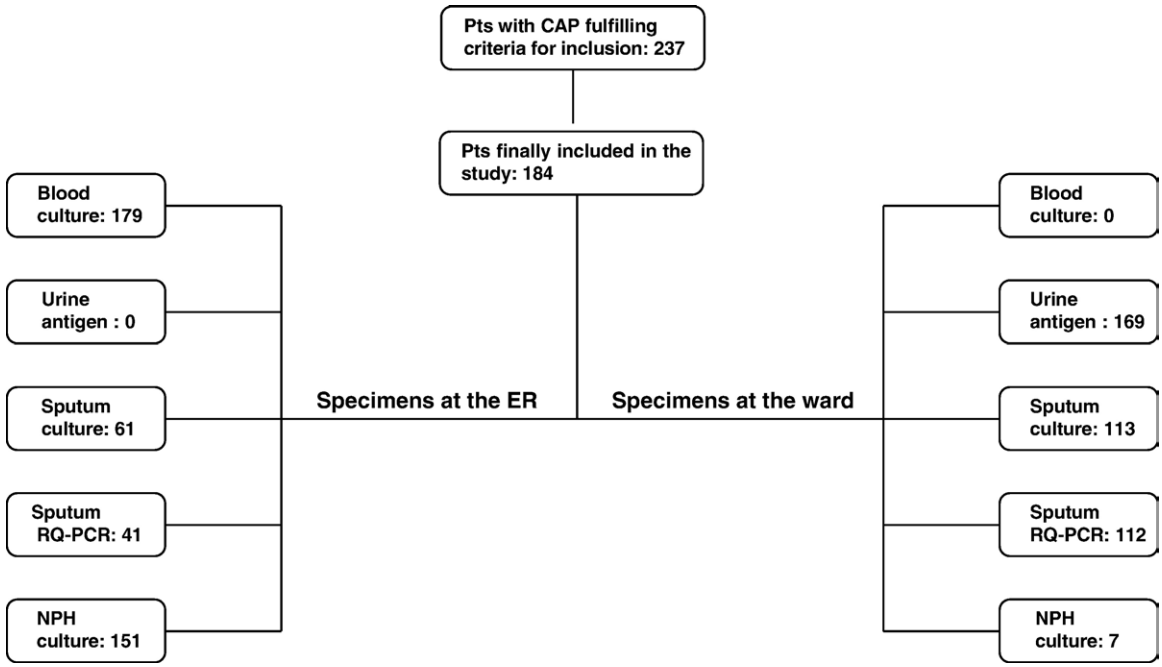

Fig. 1. Study flowchart: number of patients with specimens collected at the ER and on the ward, respectively, analyzed with different methods.

rapid methods to determine a pneumococcal etiology have been limited to Gram stain and polysaccharide antigen detection, and a clear need for improved diagnostic testing in CAP, most likely using molecular methodology rather than culture, has recently been recognized by the US National Institutes of Health (Mandell et al., 2007). In the last few years, PCR has been adopted to detect airway pathogens. The main challenge with regular PCR methods from airway samples has been the difficulties in distinguishing colonization from infection because these assays usually do not generate quantitative data (Murdoch, 2004). This obstacle could potentially be overcome by the use of a quantitative PCR assay. In the last few years, several studies have shown promising results in detecting *S. pneumoniae* from lower respiratory tract secretions by real-time quantitative PCR (RQ-PCR) (Apfalter et al., 2005; Greiner et al., 2001; Kais et al., 2006; Saukkoriipi et al., 2004; Yang et al., 2005).

The aim of this prospective study was to estimate the accuracy of a recently developed RQ-PCR method for identification of pneumococci in sputum, among patients with CAP treated in hospital.

2. Materials and methods

2.1. Patients

All patients with CAP admitted to the Department of Infectious Diseases at Karolinska University Hospital in Solna, Stockholm, Sweden, from September 13, 2004, to September 12, 2005, were reviewed for inclusion in a prospective study. A total of 184 patients were included in the study.

CAP was defined as clinical signs of acute lower respiratory tract infection with onset before admission and presence of new infiltrates on a chest radiograph. All patients were assessed by 1 of the study investigators (NJ, JH, and MK) before inclusion. This assessment took place only during daytime on weekdays, but most patients were included within 24 h of hospital arrival and in no case more than 3 days after admission to hospital. Written informed consent was obtained from all enrolled patients or from the nearest relative. The study was approved by the regional ethics committee, Stockholm, Sweden.

Patients excluded were 1) those who had been admitted to hospital for pneumonia within a month prior to the current admission, 2) those who had been admitted to hospital for any reason within 7 days before being considered for inclusion in the study, and 3) patients whose therapy had been initiated at another department.

During the study period, 242 patients who fulfilled the criteria for inclusion were admitted to the Department of Infectious Diseases. Of these, 189 patients were enrolled in the study. Fifty-three patients were not included due to the following reasons: 8 patients declined to participate in the study; 1 patient could not give informed consent because of neurologic disease, and relatives were not available; 6 patients were admitted during a long weekend and could not be included within 3 days. In 13 cases, more than 3 days after admission elapsed before the diagnosis pneumonia could be confirmed, and a further 22 patients with possible CAP were discharged before the study investigators had seen the patient; 3 patients were lost when directly admitted from the emergency room (ER) to the intensive care unit (ICU). Five patients were excluded after enrolment because the