A PCR–restriction fragment length polymorphism assay to genotype human metapneumovirus

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ABSTRACT

Human metapneumovirus (hMPV) genotypes A and B show epidemiological and probably clinical differences. This report describes a fast and simple PCR–restriction fragment length polymorphism (PCR-RFLP) assay, involving digestion of the fusion protein gene with Tsp509I, that allows lineages A1, A2, B1 and B2 to be distinguished. The assay should help in elucidating the epidemiology of hMPV, and possibly in predicting the severity of clinical infection.

Keywords Fusion protein gene, genotypes, human metapneumovirus, identification, lineages, PCR-RFLP

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Human metapneumovirus (hMPV) was first discovered in The Netherlands in 2001 [1]. This virus causes respiratory tract infection worldwide and affects individuals of all ages. The severity of episodes varies from mild upper respiratory symptoms to severe infection. Diagnosis of hMPV is usually performed using nucleic acid amplification techniques, as virus isolation is time-consuming and difficult and there are no standardised serological tests. The technique used most commonly involves RT-PCR with primers targeting various hMPV genes, e.g., the fusion, polymerase, phosphoprotein or nucleoprotein genes. Genetic analysis of the fusion (F) gene and the attachment (G) gene have been used to classify hMPV into two major genotypes, A and B, each of which is subdivided into two lineages, A1, A2, B1 and B2 [2]. The same genotyping groups have also resulted from analysis of the nucleoprotein, phosphoprotein and small hydrophobic protein genes [3,4].

Phylogenetic studies to distinguish among the different genotypes are performed by sequencing a specific gene. Although sequencing is the reference technique, this approach is expensive, time-consuming and not available in all laboratories. The present study describes a fast, simple and accessible method to identify hMPV genotypes. The method involves performing a PCR to amplify the fusion gene, using the primers and conditions described by Kaida et al. [5], followed by an analysis of restriction fragment length polymorphisms (RFLPs) using Tsp509I (New England Biolabs, Ipswich, MA, USA). The Tsp509I enzyme was selected following analysis of published gene sequences of representative lineages of hMPV in GenBank (accession nos AY145294 and AF371337, AY297749 and AY145301, AY530095, AY145295 and AY525843, and AY304362, for lineages A1, A2a, A2b, B1 and B2, respectively).

Until 2004, RNA from nasopharyngeal aspirates was extracted using a phenol–chloroform method (TRIzol LS Reagent; Gibco Life Technologies, Gaithersburg, MD, USA) according to the manufacturer’s instructions. From 2004 onwards, RNA was extracted using an automated Birobot M48 (Qiagen, Hilden, Germany) and the MagAttract Virus Mini M48 kit (Qiagen). RNA was then transcribed to cDNA using reverse transcriptase M-MuLV enzyme (USB Corp., Cleveland, OH, USA) and random primers. Aliquots of the cDNAs obtained were frozen at –80°C until PCR-RFLP was performed.

In total, 136 cDNA samples (positive for hMPV according to PCR of the F gene) were thawed and analysed by PCR-RFLP (11, 11, 12, 31, 36 and 35 samples from 2002, 2003, 2004, 2005, 2006 and 2007, respectively). These samples were obtained from >300 children who suffered an episode of acute respiratory tract infection in which hMPV was detected during the period 2002–2007. The responsible genotypes had been determined previously
by sequencing of the amplicon of the F fragment (348 bp) in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), followed by comparison with sequences at the BLAST website (http://www.ncbi.nlm.nih.gov/BLAST). Of the samples investigated, 15 were genotype A1, 74 were genotype A2, 35 were genotype B1, and 12 were genotype B2.

Genotyping by PCR-RFLP was performed by digesting 8-μL aliquots of the F fragment amplicon with 15 U of Tsp509I for 1 h at 65°C to differentiate between genotypes A and B, and among lineages A1, A2, B1 and B2. Fragments were visualised in agarose 3% w/v gels after staining with ethidium bromide.

Digestion with Tsp509I resulted in two fragments of 102 and 246 bp for hMPV lineage A1, two fragments of 76 and 219 bp for hMPV lineage A2, three fragments of 77, 116 and 153 bp for hMPV lineage B1, and two fragments of 153 and 193 bp for hMPV lineage B2 (Fig. 1).

There was full agreement between the two genotyping methods, sequencing and PCR-RFLP for 133 of the 136 samples. For three samples, the pattern obtained after PCR-RFLP analysis could not be allocated to any of the four lineages of hMPV; according to sequencing, two of these samples belonged to lineage B1 and the other to lineage B2. Thus, using Tsp509I, it was possible to distinguish clearly between the two major genotypes of hMPV, A and B, and among lineages A1, A2, B1 and B2, for >95% of the strains.

The epidemiology of the infections caused by the two genotypes of hMPV varies. There are several questions concerning the epidemiology and the clinical significance of the different genotypes of hMPV that require further investigation. The prevalence of infections caused by the two hMPV genotypes varies among geographical regions. In northern Spain, all four lineages were circulating in the 2005–2006 season, as was the case in Japan during 2001–2003 [5]. Mackay et al. [6] found that all four lineages circulated annually in Australia, with a single, usually different, hMPV lineage predominating each year. Within lineage A2, Huck et al. [7] identified two new clusters, designated A2a and A2b, and, with few exceptions, strains that were genotyped as A2 from various countries belonged to subgroup A2a. The present study revealed that Tsp509I PCR-RFLP could also be used to differentiate between clusters A2a and A2b. Samples of hMPV A2a cDNA yielded the two fragments described above for lineage A2, but hMPV A2b samples generated only one fragment of 295 bp (Fig. 1). Esper et al. [8] suggested that the CAN98-75 strain (genotype B2) can cause severe illness. However, in young children, hMPV genotype A has been reported to cause more severe disease than hMPV genotype B [9]. Although not frequent, re-infection with different genotypes can occur [10], suggesting that infection with one genotype does not protect against further infections with the other genotype.

To increase our knowledge concerning the epidemiology of hMPV infections, simple and accurate methodologies will need to be available in most laboratories. The availability of a simple, fast and economical method for genotyping a large number of hMPV samples at the same time, e.g., the PCR-RFLP assay described in the present study, would be useful for epidemiological studies and, possibly, for predicting severity of illness in patients infected with hMPV.

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REFERENCES

ABSTRACT

This study analysed the time-trends for bacteria associated with nosocomial lower respiratory tract infections (LRTIs), bloodstream infections (BSIs) and urinary tract infections (UTIs) that were reported to the German Nosocomial Infection Surveillance System for intensive care units (ICUs). Data concerning 19 822 nosocomial infections were submitted by 139 ICUs between 2000 and 2005. There was a significant increase in the proportion of Gram-negative bacteria causing LRTIs (from 63.9% to 68.4%) and UTIs (from 65.3% to 68.6%). The proportion of BSIs caused by Gram-negative bacteria declined significantly, from 36.4% to 22.7%. The frequency of methillin-resistant \textit{Staphylococcus aureus} among all \textit{S. aureus} isolates increased from 19.8% to 37.2%.

Keywords German Nosocomial Infection Surveillance System, intensive care units, methillin-resistant \textit{Staphylococcus aureus}, nosocomial infections, surveillance, time-trends

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Knowledge concerning the distribution of bacteria as causative agents of infections, together with their resistance patterns, is essential for selecting appropriate empirical antibiotic treatment. Although the microbial aetiologies of nosocomial infections (NIs) are known to differ among hospitals, or even among departments of the same hospital [1,2], new developments in healthcare systems and new treatment strategies might affect the distribution of bacterial pathogens on a wider level. Changes in the distribution of bacterial species, as well as increasing resistance rates over time, have been reported with respect to NIs in the USA [3]. The aim of the present study was to investigate the time-trends in bacteria associated with NIs in intensive care units (ICUs) in Germany between 2000 and 2005, and to determine the prevalence of resistance among selected bacterial pathogens.

The database of the German Surveillance System for NIs in ICUs (ICU-KISS) was analysed to determine the distribution of bacteria associated with nosocomial lower respiratory tract infections (LRTIs, i.e., pneumonia and bronchitis),

RESEARCH NOTE

Time-trends for Gram-negative and multidrug-resistant Gram-positive bacteria associated with nosocomial infections in German intensive care units between 2000 and 2005

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