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Original article

Diagnosis of community-acquired acute respiratory illness: From conventional microbiological methods to molecular detection (multiplex)

Diagnostic des infections respiratoires aiguës communautaires : des méthodes microbiologiques conventionnelles à celles moléculaires (multiplex)

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ABSTRACT

Investigations of the etiologic agents of community-acquired acute respiratory illness may lead to better treatment decisions and patient outcomes. In a routine care setting, we assessed the diagnostic performance of a multiplex PCR assay with respect to conventional microbiological methods, in a continuous series of adult cases of community-acquired acute respiratory illness. We enrolled 279 adult patients hospitalised for community-acquired acute respiratory illness at Tours University Hospital during the winter of 2011–2012. Respiratory samples (mostly nasopharyngeal aspirates) were studied prospectively by indirect immunofluorescence assay and multiplex PCR, that enable detection of 8 viruses and 21 respiratory pathogens respectively. In total, 255 of the 279 (91.4%) samples had interpretable results by both methods. At least one respiratory pathogen was detected by multiplex PCR in 171 specimens (65%). Overall, 130 (76%) of the 171 positive samples were positive for only one respiratory pathogen, 37 (22%) samples were positive for two pathogens and four (2%) were positive for three pathogens. With indirect immunofluorescence assay, a respiratory virus was detected in 27 of the 255 (11%) specimens. Indirect immunofluorescence assay detected some of the influenza virus A (15/51, 29%) infections identified by multiplex PCR and some (7/15, 47%) human metapneumovirus and (5/12, 42%) respiratory syncytial virus infections, but it did not detect all the adenovirus infections. Thus, access to multiplex molecular assays improves the diagnostic spectrum and accuracy over conventional methods, increasing the frequency of identification of the respiratory pathogens involved in community-acquired acute respiratory illness.

Mots clés :
Infection respiratoire
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RÉSUMÉ

La prise en charge des patients présentant une infection respiratoire aiguë communautaire peut être améliorée lorsque l'agent étiologique est identifié. Les performances diagnostiques d'une PCR multiplex ont été comparées aux méthodes microbiologiques conventionnelles sur une série continue d'adultes présentant une infection respiratoire aiguë communautaire. Durant l'hiver 2011–2012, 279 adultes avec une infection respiratoire aiguë communautaire et hospitalisés au centre hospitalier universitaire de Tours ont été inclus. Les prélèvements respiratoires, principalement des aspirations naso-pharyngées, ont été analysés prospectivement avec une technique d'immunofluorescence indirecte et de PCR

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multiplex, permettant de détecter respectivement 8 virus et 21 pathogènes respiratoires. Au total, pour 255 des 279 (91,4 %) échantillons, les résultats étaient interprétables avec les deux techniques. Au moins un pathogène respiratoire a été détecté grâce à la PCR multiplex dans 171 échantillons (65 %). Parmi ces échantillons, 130 (76 %), 37 (22 %) et 4 (2 %) échantillons étaient respectivement positifs pour un, deux et trois pathogènes. Avec la technique d'immunofluorescence indirecte, un pathogène respiratoire a été détecté dans 27 des 255 échantillons (11 %). Seulement certaines infections détectées par la PCR multiplex ont été identifées avec la technique d'immunofluorescence indirecte : virus influenza A (15/51, 29 %), metapneumovirus (7/15, 47 %) et virus respiratoire syncytial (5/12, 42 %). Aucune des infections à adenovirus n'a été détectée avec cette technique. Comparé aux méthodes de diagnostic conventionnelles, l'accès à une technique de PCR multiplex augmente la fréquence de détection de pathogènes respiratoires impliqués dans les infections respiratoires aiguës communautaires.

1. Introduction

Acute respiratory tract infections are a major cause of morbidity and mortality worldwide. They are caused by a diverse range of viruses and bacteria. Establishing a rapid etiological diagnosis of community-acquired acute respiratory illness (CARTI) may improve treatment decisions and patient outcomes. Selection of the most appropriate testing method and of the pathogens to be investigated remains a challenge [1]. A combination of several microbiological methods is often required: bacterial or viral culture, bacterial or viral antigen detection, nucleic acid amplification techniques and serology. The investigation of all potential causal agents rapidly becomes too time-consuming and costly. Choices as to the diagnostic methods to be used must therefore be made, and these choices are often driven by clinical presentation [2]. However, in clinical practice, symptoms, such as fever and myalgia, and even lung imaging findings are frequently misleading. The H1N1pdm09 flu epidemic showed that hospitalised cases of influenza-like illness were frequently attributed to a wide range of respiratory viruses and bacteria [3]. The confusion of these infections with true influenza hampered preventive and treatment measures and resulted in the unnecessary occupation of hospital beds by patients that did not actually require hospitalisation.

In recent years, multiplex RT-PCR methods have been developed, with the aim of detecting a large panel of respiratory pathogens in a single sample [4]. These techniques have been shown to be the least costly strategy, generating significant savings for hospitals [5]. In this study, we compared, in a routine care setting, the diagnostic efficacies of a multiplex PCR assay and conventional microbiological methods, in a continuous series of adult cases of CARTI.

2. Materials and methods

2.1. Ethics statement

We carried out a non-interventional study, with no addition to the usual procedures. Biological material and clinical data were obtained only for standard bacterial and viral diagnosis in accordance with doctors' prescriptions (no specific sampling, no modification of the sampling protocol, no additional questions). Data analyses were carried out with an anonymized database. According to French Public Health laws (CSP Art L1121-1.1), such a protocol does not require the approval of an ethics committee and is exempted from informed consent application.

2.2. Patients and samples

We enrolled a continuous series of 279 adult patients hospitalised for CARTI at Tours University Hospital during the winter of 2011–2012 (from week 47 in 2011 to week 18 in 2012). The mean age of the patients was 61 years (range: 15–95 years). The sex ratio (M/F) was 1.30. In total, 167 patients were hospitalised in the intensive care unit (ICU) and 112 in medical wards. Most of the samples studied were nasopharyngeal aspirates (n= 235). For some patients, only bronchoalveolar lavages (n= 42) or sputum samples (n= 2) were obtained.

2.3. Detection of respiratory viruses

2.3.1. Antigen detection by indirect immunofluorescence assay

Bronchial cells obtained from nasopharyngeal aspirates by centrifugation were suspended in buffer and spotted onto slides, then air-dried, fixed in acetone and incubated for 15 minutes with a specific mouse monoclonal antibody (Argène bioMérieux, France). The slides were washed and incubated with goat anti-mouse fluorescein-conjugated monoclonal antibodies for 15 minutes. The slides were then washed again and examined under a fluorescence microscope. We analysed only samples containing at least 20 bronchial cells per spot. All samples were tested for influenza virus A (INF A), influenza virus B (INF B), adenovirus (AdV), human metapneumovirus (HMPV), parainfluenza viruses (PIV) 1 to 3 and respiratory syncytial virus (RSV).

2.3.2. Multiplex real-time PCR

Total nucleic acids were extracted with an EZ1 Advanced XL automatic extractor (Qiagen, France), according to the manufacturer's instructions, beginning with 200 μl of each respiratory specimen. The final elution volume was 90 μl.

Samples were analysed with Respifinder22, (Pathofinder, The Netherlands), a multiplex molecular assay for the detection of 18 respiratory viruses (AdV, human bocavirus (HBoV) human coronaviruses (HCoV) NL63, OC43, 229E, HKU1, HMPV, INF A, INF B, INF A-H1N1pdm 2009, PIV 1 to 4, RSV A, RSV B, rhinovirus/enterovirus (HRV/EV) and four bacteria (*Bordetella pertussis, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila*). Assays were performed on a LightCycler 480 (Roche) according to the manufacturer's instructions.

2.3.3. M. pneumoniae *serology*

IgG and IgM antibodies were detected with the alphaWell *M. pneumoniae* ELISA kit (Mikrogen Diagnostik, Germany), according to the manufacturer's instructions.

3. Results

Sixteen of the 279 (6%) samples could not be studied with the multiplex assay because they contained RT-PCR inhibitors. At least one respiratory pathogen was detected by multiplex PCR in 171 of the 263 (65%) specimens analysed. Multiple infections were observed in 41 samples (37 with two pathogens and four with three pathogens). Thus, in total, 195 viruses and 21 bacteria were detected (Table 1). The percentage of specimens testing positive varied from 74% (January 2012) to 44% (March 2012); peak incidence was at week 8, when 90% of samples tested positive. INF A (n = 50) was the most common etiologic agent detected, followed by HRV/EV (n = 35) and HBoV (n = 34). Eight pathogens accounted for more than 5% of the total (AdV, HBoV, HCoV OC43, HMPV, INF A, HRV/EV, RSV A and B, *M. pneumoniae*). AdV, HBoV and HRV/EV were observed throughout the study period. The incidence of influenza virus infections peaked during weeks 8 to 11. HCoV OC43, HMPV and RSV and *Mycoplasma* infections were more evenly distributed over the winter months (Fig. 1). We observed no difference in the distribution of pathogens between ICUs and medical wards or between male and female patients (data not shown). Only *M. pneumoniae* infections were significantly more frequent in younger patients. The mean age of the patients infected with *M. pneumoniae* was 47 years, whereas the mean age of