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Surveillance of upper respiratory infections using a new multiplex PCR assay compared to conventional methods during the influenza season in Taiwan

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ABSTRACT

Objectives: To improve diagnosis as part of laboratory surveillance in Taiwan, influenza-like illness (ILI) surveillance was conducted using a new multiplex PCR assay (FilmArray) and the results compared to those of conventional methods The study was performed during the winter months.
Methods: Throat swabs from patients with an ILI presenting to physicians in sentinel practices were collected during the 2016–2017 influenza season.
Results: A total of 52 samples tested positive by FilmArray Respiratory Panel. Forty percent were influenza A virus, and subtype H3N2 virus was the major epidemic strain. However, nearly 60% of ILI cases seen at sentinel sites were caused by non-influenza pathogens. The results of the FilmArray assay and cell culture were identical, and this assay was more sensitive than a rapid influenza diagnostic test. Genetic analyses revealed new influenza A H3N2 variants belonging to a novel subclade 3C.2a2.
Conclusions: The FilmArray assay facilitates urgent testing and laboratory surveillance for common viral and bacterial respiratory pathogens. This study demonstrated the use of a highly sensitive assay using clinical samples that is feasible for application worldwide. This may lead to an increased rate of diagnosis of viral infections and to improved patient outcomes, and in particular to a reduction in the overuse of antibiotics and antivirals.

Introduction

Acute respiratory infections are often caused by viruses and are an important cause of morbidity and mortality in Taiwan (Chang et al., 2016; Chiu et al., 2014; Kim et al., 2003; Tsai et al., 2001). During past influenza seasons, many severe cases and deaths were reported among the middle-aged population group, which had a major socioeconomic impact. Therefore, the Taiwan Centers for Disease Control (CDC) expanded their scope to provide free influenza vaccinations for children aged 6 months to <18 years, pregnant women, women within 6 months of giving birth, adults aged >50 years, at-risk individuals with chronic medical conditions, individuals with rare diseases or major illnesses/injury, health care and public health personnel, poultry farmers and animal health inspectors, individuals with a body mass index (BMI) of ≥30 kg/m^2, and residents and personnel at nursing institutes. This campaign increased the flu vaccination coverage from 13% to 25% of the total population of Taiwan.

However, clinical signs and symptoms of upper respiratory infections are similar for many different viruses, making an etiological diagnosis based on clinical presentation alone difficult and sometimes leading to delays in therapeutic management. The ability to detect viruses has improved with the availability of PCR,

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and several new viruses have been identified (Poutanen et al., 2003; van den Hoogen et al., 2001; Zaki et al., 2012). The use of more sensitive methods has led to an increased rate of diagnosis for viral infections and to improvements in patient outcomes. With an automated multiplex PCR device, the FilmArray Respiratory Panel, 17 viral pathogens and 3 bacterial pathogens can be detected in a closed system that requires only 3 min of hands-on time and approximately 1 h of instrumentation time.

The aim of this study was to determine whether the results provided by this new multiplex PCR technique would be useful for application in public health laboratories for the surveillance of influenza-like illness (ILI).

Materials and methods

Samples

Throat swabs were collected from patients with an ILI during the influenza season November 2016 to January 2017 in Taipei, Taiwan. Taipei City is the capital city and accounts for approximately one tenth of the total population of Taiwan. ILI was defined as the sudden onset of fever (≥38 °C) and a cough and/or a sore throat without a known cause other than influenza. The biological materials obtained in this study were used for standard diagnostic procedures as requested by the patient's physician; no specific sampling method was used and no modifications were made to the sampling protocols. In accordance with local regulations, the procedure did not require specific patient consent.

Conventional culture and PCR

Conventional methods were used for the clinical samples. Samples were inoculated in MDCK, MK2, HEp-2, RD, MRC-5, Vero, A549, and HeLa cell lines, and cell growth was evaluated in MEM (Minimum Essential Medium) containing 2% (v/v) fetal bovine serum and antibiotics in a 5% CO_2 humidified incubator for 7–10 days, or until a cytopathic effect (CPE) was observed. An indirect immunofluorescence assay (IFA) using monoclonal antibodies (Merck Millipore Cat. Nos. 3105 and 3360) to influenza A and B viruses, parainfluenza virus types 1–3, respiratory syncytial virus (RSV), adenovirus, and enterovirus was also performed. Furthermore, conventional PCR was used to detect rhinovirus, enterovirus, coronavirus, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*, as reported in previous studies (Freymuth et al., 1999; Rose et al., 2003).

Multiplex PCR

Clinical samples were analyzed using the BioFire FilmArray Respiratory Panel (BioFire Diagnostics, Salt Lake City, UT, USA). This panel was used in a multiplex PCR assay that detects 20 respiratory pathogens, including RSV, influenza A virus H1, influenza A virus H1 2009, influenza A virus H3, influenza B virus, adenovirus, parainfluenza virus types 1–4, human rhinovirus (HRV)/human enterovirus (HEV), human metapneumovirus, human bocavirus, human coronavirus types OC43, 229E, NL63, and HKU1, *Bordetella pertussis*,*Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* (BioFire Diagnostics, 2013; Poritz et al., 2011).

Phylogenetic characterization

Pairwise alignment was performed using BioEdit 7.2.5 (Hall, 1999), while multiple sequence alignment was performed using MUSCLE 3.8 (Edgar, 2004), where the aligned sequences were further manually inspected and edited. Phylogeny reconstruction and evaluation were inferred with the maximum likelihood method in MEGA 6.0.6 (Tamura et al., 2013), using the transition/transversion ratio and alpha parameter of the gamma distribution estimated by maximum likelihood with TREE-PUZZLE software (Schmidt et al., 2002). The robustness of the maximum likelihood trees was evaluated statically by bootstrap analysis with 1000 bootstrap samples. The genomic sequences of vaccine strains recommended by the World Health Organization (WHO) were used as reference sequences and were retrieved from the National Center for Biotechnology Information (NCBI) Influenza Virus Resource (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html) and the Global Initiative on Sharing All Influenza Data (GISAID, http://www.gisaid.org).

Nucleotide sequence accession numbers

The hemagglutinin nucleotide sequences of the 23 influenza A viruses isolated in this study have been deposited in the GISAID database and assigned accession numbers **EPI944520**–**EPI944542**.

Results

Surveillance

From November 2016 to January 2017, a total of 60 samples from three sentinel sites in Taipei City were collected and sent to the Taiwan CDC. Among the subjects, 27 (45%) were male and 33 (55%) were female, and they ranged in age from 1 to 96 years. The most common clinical manifestation was fever >38.5 °C. Overall, a total of 52 samples were positive for respiratory pathogens; no dual infections were found in any patient. Of these 52 samples, 24 (40%) were influenza A-positive (23 cases of A/H3 and one case of A/H1). The other non-influenza viruses were RV/HEV (*n*=13, 21.6%), parainfluenza virus (*n*=3, 5%), human coronavirus OC43 (*n*=1, 1.7%), *Mycoplasma pneumoniae* (*n*=2, 3.3%), human metapneumovirus (*n*=1, 1.7%), and human adenovirus (*n*=5, 8.3%) (Table 1).

Table 1
Age distribution of the patients and clinical identification of respiratory pathogens during the 2016–2017 influenza season, Taiwan.

	Patients (*n* = 60)		Symptoms	Clinical identification							
	Male	Female	Fever (>38.5 °C)	Influenza A virus	Adenovirus	Parainfluenza virus	Human rhinovirus/ enterovirus	Human metapneumovirus	Human coronavirus OC43	RSV	*Mycoplasma pneumoniae*
Age group	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
0–5	2 (3.3)	7 (11.6)	9 (15.0)	2 (3.3)	1 (1.7)	1 (1.7)	3 (5.0)	1 (1.7)			
6–18	19 (31.7)	14 (23.3)	33 (55.0)	14 (23.3)	3 (5.0)	2 (3.3)	8 (13.3)			2 (3.3)	
19–24	2 (3.3)	4 (6.6)	4 (6.6)	1 (1.7)					1 (1.7)		2 (3.3)
25–49	3 (5.0)	6 (10.0)	9 (15.0)	6 (10.0)	1 (1.7)		1 (1.7)			1 (1.7)	
50–64	0 (0.0)	2 (3.3)	2 (3.3)				1 (1.7)				
65+	1 (1.7)	0 (0.0)	1 (1.7)	1 (1.7)							
Total	27 (45.0)	33 (55.0)	58 (96.6)	24 (40.0)	5 (8.3)	3 (5.0)	13 (21.6)	1 (1.7)	1 (1.7)	3 (5.0)	2 (3.3)

RSV, respiratory syncytial virus.