Multiplex tests for respiratory tract infections, direct utility of FilmArray respiratory panel in the emergency department

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Abstract

Background

The FilmArray respiratory panel with 20 targets for viral and atypical pathogens aimed at point of care tests, as multiplex tests for respiratory tract infections it has been widely used and verified in various clinical trials. The FilmArray respiratory panel (FilmArray RP) seems to be a reliable and rapid tool in special tests settings such as clinical laboratory, and it’s necessary to evaluate the panel’s performance in point of care directly with non-specific tests settings.

Methods

In this single-centre, open, observational study, we enrolled outpatients with respiratory tract infection from emergency department during 2016-2017 winter season, specimens from participants were detected using FilmArray RP. All steps including recruitment, collection of specimens and testing were performed by our clinical staff. The results of pathogens detected, clinical and demographic data were collected and analyzed.

Results

Between October 2016 and May 2017, we enrolled 271 eligible patients, FilmArray RP was successful used in 270 patients: 196 (72.6 %) patients had been detected with one or more pathogens, 74 (27.4) patients got negative results; in the analysis of signal pathogen detected, influenza A virus had the highest rate of detection, and the rate in group ≤ 16 years, 16-49 years and ≥ 50 years was 22.8 %, 32.4 %, 31.1 % respectively; 45 (16.7 %) patients were co-detected with two or more respiratory pathogens, most multiplex pathogens were rhinovirus/enterovirus co-detected with *Bordetella. pertussis* (17.8 %). The outcomes provided by FilmArray RP had impact on the prescriptions of antimicrobials, there were difference in the rates of antibiotic prescriptions and anti-influenza prescriptions among patients with Flu A/B virus detected, non-Flu A/B virus pathogen detected and non-pathogen.

Conclusions

The performance of FilmArray RP using by clinical staffs was successfully implemented in emergency department for which is the first time in China. The multiplex diagnostic system significantly

increased the rate of respiratory pathogens detected in diagnostic methods limited hospital. Despite this, FilmArray RP has a potential use as a tool for point of care testing in non-specific setting and operated by clinical staffs not in a laboratory.

Background

Respiratory tract infections (RTI) are a significant cause of morbidity and mortality worldwide, particularly during winter months with seasonal respiratory epidemics [1-4]. More than the morbidity and mortality associated with RTI, the infections also represent a huge impact on emergency department (ED) visits, outpatient medical visits, hospital admissions and burden of antimicrobial prescriptions, especially in developing countries because of absence of rapid and accurate laboratory-depended testing for respiratory pathogens [5-7]. The majority of RTI are caused by respiratory viruses followed by infections of bacterial pathogens [8, 9]. A principal challenge for clinical staffs to distinguish of RTI is that the infections have similar symptoms and clinical signs, such as to identify influenza from “common cold” or bacterial community-acquired pneumonia (CAP) [10]. It is usually initiated empiric treatment with antibiotics to patients suspected with viral infection, inappropriate prescriptions of antibiotics may result in extra costs, potential challenge of bacterial resistance and even environmental pollution [11, 12]. Definitive etiological diagnosis based on rapid testing and the ability of distinguish multiple respiratory pathogens may improve this unreasonable situation.

To compare to traditional tests such as direct fluorescence assays (DFA) and cell culture, laboratory-developed polymerase chain reaction (PCR) tests have highly sensitivity and specificity for the detection of respiratory pathogens with shorten turn-around time (TAT) [13]. The FilmArray Respiratory Panel (BioFire Diagnostics; Salt Lake City, UT, USA) is an automated multiplex molecular system for the detection of 20 viruses, 2 atypical pathogens and a bacterium in 65 min. The evidence for improved influenza detection and antimicrobial prescriptions and reduced length of stay of this system has been widely proved by many clinical trials [1, 14-18]. As an automated multi-pathogen detection system, FilmArray RP is an integrated diagnostic system which has the feature of rapid, accurate and reproducible, in additional the system minimizing the needs for professional lab facilities and trained technicians [19]. Though being defined as multi-pathogen molecular detection of point of

care (POC) tests, the operators of this system from pouch preparation to data analysis trend to be experienced laboratory staffs in previous published articles, and a general laboratory or microbiology laboratory in hospital is the only place of this multi-pathogen tests. Thus, to understand the direct utility of FilmArray RP as POC tests in outpatients is an important work.

In this study we undertook a describe the direct utility of FilmArray RP as POC tests in our emergency department which is special for infectious diseases during winter months with seasonal respiratory epidemics with ED visitors. The aim of this study was to investigate the performance of the FilmArray RP system when serviced for non-specific tests setting and with clinical staffs' hand on.

Methods

Study design and participants

This single-centre, open, observational study took place between October 2016 to May 2017 in Beijing Ditan Hospital (BJDH), affiliated to Capital Medical University, China. BJDH is a teaching hospital with 800 beds. As infectious diseases specialized hospital, we have set up separate ED for patients with infectious disease and a large proportion of patients come for this ED for RTI in winter. Participants were enrolled from the ED in BJDH. Included patients were fever or feeling feverish, cough, runny nose or stuffy nose, fatigue or tireless, could be recruited directly of first arrival at the ED, possibly present with vomiting or diarrhea. Patients were excluded if they were fever longer than 7 days, C-reactive protein (CRP) > 40 mg/L. We also excluded patients with unconsciousness, HIV-infected, treated with immunosuppressive therapy at that moment. A written informed consent was obtained by emergency department staff from participants, child's parents or guardians. This study was approved by the ethics committee of BJDH.

Clinical staff training

FilmArray RP was the first multiplex test implemented in the ED by clinical staffs, so all clinical staffs were educated about the manufacturer's instructions by microbiology staff and personnel training of operate the FilmArray instrument was followed. Clinical staffs collected all the respiratory samples consulted the standard operating procedure of clinical samples collection used in our hospital. Before the October initial of the study, all clinical staff's competency evaluations were totally completed.

Sample collection and FilmArray RP assay

For FilmArray RP assay, the nasopharyngeal swab for respiratory sample was collected using swab with nylon flocked (Copan Diagnostics, Italy) at mid-turbinate which would be placed in viral transport media (VTM, Copan Diagnostics) immediately. Specimens in VTM were tested when clinical staff received in the ED. Before the establish of the FilmArray system, gold-labeled antigen staining was used for influenza A virus and influenza B virus, there is no other rapid/POC testing for respiratory viral pathogens at our hospital. The physicians of the ED decided on make a antibiotics prescription according patient's clinical manifestation, results of laboratory examination and FilmArray RP assay. Trained assistant collected all clinical data and etiological information from a standard case report form and input into an electronic study database.

In this study, the FilmArray assay (FilmArray RP panel, BioFire Diagnostics, LLC, Salt Lake City, Utah) detects 20 targets of viral and atypical pathogens present in respiratory samples, in which including 17 respiratory viruses for rhinovirus/enterovirus (Rhino/Entero), respiratory syncytial virus (RSV), adenovirus (AdV), human metapneumovirus (hMPV), influenza B virus (Flu B), influenza A virus unsubtyped/H1/H3/2009H1 (Flu A/unsubtyped, Flu A/ H1, Flu A/H3, Flu A/2009H1), coronavirus 229E/HKU1/NL63/OC43 (Cov 229E, Cov HKU1, Cov NL63, Cov OC43), parainfluenza 1/2/3/4 (PIV 1, PIV 2, PIV 3, PIV 4), one bacterium is *Bordetella pertussis* (*B. pertussis*) and two atypical pathogens include *Chlamydia pneumoniae* (*C. pneumoniae*) and *Mycoplasma pneumoniae* (*M. pneumoniae*)[20]. Rehydration buffer (1.0 ml) and the VTM (200 μl) were loaded into the testing pouch by clinical staff under a ventilation hood. After scanning the sample identity document, automatic nucleic acid extraction, RNA reverse transcription, the first-stage and second-stage multiplex PCR amplification was started successively and the result was available in 65 min. For each target, the second- stage PCR amplification was designed in triplicate wells. The FilmArray assay automatically performs the melting curve of the second- stage PCR to provide the results as positive or negative, if internal control amplifications reported a negative result, the assay is defined as "Invalid" with no pathogens result.

Statistical analysis

Patient demographic characteristic was collected, including age, gender. Clinical syndrome was included fever, cough, during of symptoms, and if complicated with pneumonia. Clinical outcomes evaluated were antibiotics prescription and anti-influenza prescription. All data management software was Microsoft Excel 2015. All analysis in this study were used IBM SPSS Statistics software v22.0. Categorical variables of demographic and clinical characteristics, clinical outcomes were shown in percentages, and compared with Pearson's Chi-square test or Fisher's exact test for differences. White cell count, level of C-Reactive protein and lymphocytes were presented as median (interquartile range). The differences of antimicrobials among groups with different pathogen detected were compared with Student's *t* test or Mann-Whitney *U* test. *P* values < 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics

Between 15th October 2016 and 31th March 2017, the study assessed 315 patients for eligibility, and 271 of them were eligible to participate, none of participants withdrew from the study (Fig. 1). Forty-four (24.0 %) of the patients were excluded for 19 were declined consent, 12 had fever longer than 7 days, 7 patients refused nasopharyngeal swab, 4 had high level of CRP and HIV is positive for 2 patients. Grouped by the result of FilmArray RP assay, the characteristics of pathogen detected group and non-pathogen detected group are shown in table 1. One hundred and ninety-six (72.6 %) patients had been detected with one or more pathogens, 93 (47.4%) patients were □ 16 years but age between 16-49 accounted for large proportion in non-pathogen detected group. There were high percentage of the clinical characteristic of fever (81.1%) and coughing (83.8) in group of non-pathogen detected versus pathogen detected group (60.7%, 61.7% respectively). The median level of White cell count and C-reactive protein was higher in the non-pathogen detected group compared to pathogen detected group (non-pathogen detected group 9.28 $\times 10^9$/L [IQR 4.05-11.86], 28.86 mg/L [IQR 7.40-34.83], pathogen detected group 8.5 $\times 10^9$/L [IQR 4.52-9.40], 16.70 mg/L [IQR 2.40-19.30]).

Analysis of signal pathogen detected

Overall, 151 (55.9%) of the 270 patients had a signal pathogen detected (Table 2). Patients with

signal pathogen detected were divided into three groups according age, children ≤ 16 years (n = 114) was the largest group in our study. The numbers of age group 16-49 and ≥ 50 years were small compared with the youngest group, with 111 and 45 patients in each group respectively.

The highest prevalence of targeted pathogens in three age groups was Flu A, the prevalence of Flu A in group ≤ 16 years, 16-49 years and ≥ 50 years was 22.8 %, 32.4 %, 31.1 % respectively. More specifically, the highest rate of Flu A detected specimens from three distinguishable subtype is FluA/H3 in three age groups, with a rate of 26.7 % in group ≥ 50 years, followed by 19.8 % in group 16-49 years and 14.0 in group ≤ 16 years. The others respiratory pathogens such as Rhino/Entero (6.3 %), RSV (6.3 %), Flu B (2.2 %), Cov (1.5 %), *M. pneumoniae* (4.1 %), *B. pertussis* (3.3 %) had low rate less than 10.0 %, and the detected rate of hMPV (0.7 %), AdV (0.7 %) and PIV (0.4 %) was less than 1.0%. However, there was no detected of *C. pneumoniae* in all 151 patients. As a common respiratory pathogen of pertussis in children, all 9 cases of *B. pertussis* were detected only in group ≤ 16 years. More detailed information is shown in Table 2.

Analysis of co-pathogens detected

Among 270 patients, 45 were co-detected with two or more respiratory pathogens, with a rate of 16.7%. Among the 45 patients with co-detected respiratory pathogens, co-detected with dual pathogens were 40 accounted for majority of the samples. Patients with triple pathogens or quadruple pathogens co-detected were 4, 1 respectively. Rhino/Entero co-detected with *B. pertussis* had the largest number of 8 (17.8 %), followed by 6 (13.3 %) cases of RSV co-detected with PIV. More detailed information is shown in Table 3.

In this 45 cases with co-detected respiratory pathogens, a total number of all nine kinds detected pathogens was 98, RSV and *B. pertussis* were the highest rate of 17.3 %, followed by Flu A (16.3 %), Rhino/Entero (15.3 %) and PIV (15.3 %). The rate of detection of AdV, hMPV, Cov and *M. pneumoniae* were less than 10.0 %, (8.2 %, 4.1 %, 5.1 %, 1.0 % respectively). *C. pneumoniae* was not detected either in this patients.

Outcome of antimicrobial prescription

According the results of FilmArray RP panel, 98, 98 and 74 patients were assigned to the Flu A/B virus

detected group, non-Flu A/B virus pathogen detected group and non-pathogen detected group respectively. Antibiotic prescription rates were significantly different among the three groups (c^2 = 37.1, $P < 0.001$), with the highest rate of 50.0 % in non-pathogen detected patients in table 4. Between patients with non-Flu A/B virus pathogen detected and those who with non-pathogen detected there was significant difference about antibiotic prescription rates (28.8 % and 51.4 %, respectively, [c^2 = 8.2, $P < 0.001$]).The difference of anti-influenza prescription rates were also significant in three group (c^2 = 98.8, $P < 0.001$), with those detected with Flu A or Flu B receiving the most prescriptions (71.6 %). Anti-influenza prescription rates were no difference between patients with non-Flu A/B virus pathogen detected and those with non-pathogen detected (3.1 % and 8.1 %, respectively, [c^2 = 1.8, $P = 0.194$]). More detailed information is shown in Table 4.

Discussion

This pragmatic, clinical trial is the first, to our knowledge, to report on the performance of FilmArray RP panel for directly using in ED for outpatients by clinical staffs, on the outcomes including analysis of signal pathogen detected, analysis of co-pathogens detected, and antimicrobial prescription rates. After

clinical staff training and personnel training, 271 specimens were detected over the winter season using the automated nested multiplex PCR system for pathogen detection, that yielded a detected rate of 72.6 % with a positive result in 270 passed specimens. Although, the PCR instrument was operated by clinical staff, only one of all samples failed. As the three groups divided by age in the analysis of signal pathogen detected, the group of ≤ 16 years had the highest positive rate of 81.6 %, followed by group of ≥ 50 years (66.7 %) and group of 16 - 49 years had the lowest positive of 65.8%. The trend of low detection rate of respiratory viral pathogens in adults is consistent with previous studies, Christine M. et al. have shown that with the increasing of age the positive rates with FilmArray RP panel are decreasing, and other studies with multiplex respiratory pathogens PCR also reported lower detected rates in the population of adults[3, 21, 22]. However, the whole detected rate in this study which resuls from the clinical staffs' operation is higher than other studies in which all

diagnosis with FilmArray RP panel, including the study of respiratory pathogens at tertiary care medical centers (age of 5 days to 91 years), ED or urgent care of pediatric patients, ward-based operating with in-patient and out-patient medical areas[7, 22-24]. It is possible that ED-based POC testing has a shorten time from sample collection to perform a FilmArray run than testing in specific tests settings. It is also possibly relating to the difference of time and sample size, the characters of age distribution of participants.

We noted the difference of detected number of respiratory pathogens in the result with a signal pathogen detected. Flu A was the most detected viral pathogen in three groups, Liu et al., Qian et al. and Jin et al. similarly observed that Flu A was the most detected pathogen in RTI in winter in their studies [16, 25, 26]. Rhino/Entero and RSV was the predominant virus for all age group but far more less than the most detected Flu A [19, 27]. Rhinovirus is a common virus of all seasons, as we all know, peaks of rhinovirus infections are well documented around September, a low positive rate of rhinovirus may be associated with the tendency of the doctors in ED to enroll patients with severe symptoms, because of the purchase price of a FilmArray RP panel is very expensive [3, 21].

M. pneumoniae was observed in three groups, and the positive rates of the children group and the old adult group are higher than that of group 16 - 49 years, in China, *M. pneumoniae* is reported as one of the most common pathogen of patients with CAP and it is an unexpected result in our study [25, 28]. With an overall detected rate of 4.1 % in the participants of 270, further revealing the diagnostic performance of FilmArray RP panel in ED's application. *B. pertussis* cases were detected only in group ≤ 16 years with a positive rate of 7.9 % in 114 patients, at present, the using of culture and serology methods in diagnosis of pertussis are the only choice in China, FilmArray RP panel may help to manage the patient with suspected pertussis since it is difficult to identify other kinds respiratory pathogens for which often cause similar clinical symptoms like pertussis [29]. No *C. pneumoniae* case was detected in our study, the location of Beijing has a lowest incidence of reported *C. pneumonia* in the entire China with an incidence of 0.40 % and 2.97 % by Chen et al. and Zhao et al. respectively [30, 31].

Respiratory pathogens more than one were co-detected in 16.7 % of the 270 specimens in this study,

unlikely, Jin et al. reported 25.5 % co-detected of patients from children aged 19 days to 15 years with RTI by FilmArray Respiratory Panel [16]. However, in adult, studies reported lower rates of approximately around 10 % and 15.9 % [19, 21]. For each Respiratory pathogen of co-detected, the largest proportion of which included RSV (17.3 %) and *B. pertussis* (17.3 %), and Flu A was the second common pathogen with a low positive rate (16.3 %) than that in the analysis of signal detected. Because of there was no laboratory testing for respiratory viral pathogens (except for Flu A and Flu B) at our hospital, the detections of multiplex respiratory organisms may be the most unexpected result for the clinical staffs in our ED, fully illustrating the value of FilmArray RP panel as a tool of POC testing in clinical department.

We found that patients whose detected with Flu A and Flu B received significantly lower antibiotic prescriptions and more anti-influenza prescriptions than who had a positive result of non-Flu A/B viral pathogen or with a negative result for all pathogens. The detection of a positive result for viral pathogen contributes to decrease the usage of antibiotic in patients with RTI in ED, which suggested that the using of FilmArray RP panel may be a good tool for reducing antibiotic prescriptions in outpatients with RTI. However, there was no evidence for significant decrease for the anti-influence prescriptions usage between non-Flu A/B viral pathogen detected patients and non-pathogen detected patients. Through without influenza A or B virus identified, the doctors were more likely to prescribe anti-influenza drugs for outpatients in winter season, this notice may be a hit for preventing inappropriate prescriptions of anti-influenza [32]. As for the effects of respiratory viral pathogens results on antimicrobial prescriptions rates including antibiotic and anti-influenza have been reported for mixed findings by previous studies, however, in our study, multiplex pathogens PCR system of FilmArray has shown the potential ability of reducing unnecessary antimicrobial prescriptions. According the results of FilmArray RP panel, 98, 98 and 74 patients were assigned to the Flu A/B virus detected group, non-Flu A/B virus pathogen detected group and non-pathogen detected group respectively. Antibiotic prescription rates were significantly different among the three groups (c^2 = 37.1, $P < 0.001$), with the highest rate of 50.0 % in non-pathogen detected patients in table 4. Between patients with non-Flu A/B virus pathogen detected and those who with non-pathogen

detected there was significant difference about antibiotic prescription rates (28.8 % and 51.4 %, respectively, [c^2 = 8.2, P < 0.001]).The difference of anti-influenza prescription rates were also significant in three group (c^2 = 98.8, P < 0.001), with those detected with Flu A or Flu B receiving the most prescriptions (71.6 %). Anti-influenza prescription rates were no difference between patients with non-Flu A/B virus pathogen detected and those with non-pathogen detected (3.1 % and 8.1 %, respectively, [c^2 = 1.8, P = 0.194]). More detailed information is shown in Table 4.

However, there are numbers of limitations in this study. First, our study was a single-centre study and results from multicentre need to be reported for verifying. Second, another possible limitation of our study is the bias from age among participants, as we all know, young children in which infected with RTI have a totally different percentages of respiratory pathogenic spectra. Third, because the high cost of FilmArray RP panel we choose to implement our study only in a single winter season and we dose not own data from a complete year. Fourth, the study was conducted in ED which is special for infectious diseases other than general ED that including clinical staffs facing more kinds of diseases. Finally, the utility of FilmArray respiratory panel in the emergency department needs further evaluation in multicentre studies and more patients in ED.

Conclusion

In this study we tested respiratory pathogens in patients with RTI in ED using FilmArray respiratory panel by clinical staffs, and this is the first time in China. We found that, FilmArray, the automated nested multiplex diagnostic PCR system was a rapid and simple tool for clinical staffs in a non-specific tests setting. The multiplex diagnostic system significantly increased the rate of respiratory pathogens detected, and clinical staff of ED performed FilmArray RP testing without too much “Invalid” results suggesting that this diagnostic system has a potential using for many divergent clinical settings. Based on the results of FilmArray RP panel, in patients with non-detected pathogens the usage of antibiotic prescriptions and anti-influenza prescriptions could reduce in the study ED.

Abbreviations

FilmArray RP: FilmArray respiratory panel; RTI: Respiratory tract infections; ED: Emergency Department; CAP: Community-acquired Pneumonia; DFA: Direct Fluorescence Assays; TAT: Turn-

around time; POC: Point of care; BJDH: Beijing Ditan Hospital; CRP: C-reactive protein; HIV: Human Immunodeficiency Virus; VTM: Viral Transport Media.

Declarations

Ethics approval and consent to participate

Before start, this study was approved by the ethics committee of Beijing Ditan Hospital with the reference 2017ZX10103004. After informed the patients that the usage of samples and the aims of detections, written informed consents were obtained from each patient.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable. All major data generated or analyzed during this study were included in this article.

Competing interests

There are no competing interests.

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Authors' contributions

YSY and WLH performed all conception and design of the study; WF, YD and GGJ organized the database and had useful suggestions on clinical significance of this case report; WLS, LD, and XDH contributed the imaging diagnosis. YSY contributed the molecular diagnostic experiments and wrote the first draft of the manuscript; WLH revised the discussion. All authors have read and approved the manuscript.

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Tables

Table 1 General characteristics of enrolled patients

	Pathogen detected	Non-pathogen detected
Male gender	53.1% (104/196)	55.4% (41/74)
No. (%) of patients of age		
≤ 16 years	93 (47.4)	21 (28.4)
16 - 49 years	73 (37.2)	38 (51.4)
≥ 50 years	30 (15.4)	15 (20.4)
No. (%) of patients of prescription during last 2 weeks		
Antibiotic used	10 (5.1)	6 (8.1)
Anti-influenza used	6 (3.1)	7 (10.8)
No. (%) of patients of fever	119 (60.7)	60 (81.1)
No. (%) of patients of coughing	121 (61.7)	62 (83.8)
Duration of symptoms (days)[a]	2.3 (1-2)	3.4 (2-3)
Complicated with pneumonia (%)	40 (20.4)	24 (32.4)
White cell count ($\times 10^9$ /L)[a]	8.5 (4.52-9.40)	9.28 (4.05-11.86)
C-reactive protein (mg/L)[a]	16.70 (2.40-19.30)	28.86 (7.40-34.83)
Lymphocytes ($\times 10^9$/L)[a]	4.64 (1.26-5.73)	3.03 (0.91-1.75)

[a] median (IQR). Duration of symptoms: the time from patients had symptoms to visit emergency department.

Table 2 Prevalence of respiratory pathogens signal detected in different age groups

Result	≤ 16 years		16 - 49 years		≥ 50 years		T
	No.	Prevalence (%, n= 114)	No.	Prevalence (%, n= 111)	No.	Prevalence (%, n= 45)	N
Not detected	21	18.4	38	34.2	15	33.3	7
Rhino/Entero	6	5.3	7	6.3	4	8.9	1
RSV	8	7.0	7	6.3	2	4.4	1
AdV	1	0.9	1	0.9	0	0.0	2
hMPV	0	0.0	2	1.8	0	0.0	2
Flu B	2	1.8	2	1.8	2	4.4	6
FluA/H1	0	0.0	0	0.0	0	0.0	0
FluA/H3	16	14.0	22	19.8	12	26.7	5
FluA/2009 H1	8	7.0	7	6.3	0	0.0	1
Flu A unsubtyped	2	1.8	7	6.3	2	4.4	1
Flu A total	26	22.8	36	32.4	14	31.1	7
Cov 229E	0	0.0	1	0.9	2	4.4	3
Cov HKU1	0	0.0	1	0.9	0	0.0	1
Cov NL63	0	0.0	0	0.0	0	0.0	0
Cov OC43	0	0.0	0	0.0	0	0.0	0
Cov total	0	0.0	2	1.8	2	4.4	4
PIV1	2	1.8	0	0.0	0	0.0	2
PIV2	0	0.0	0	0.0	0	0.0	0
PIV3	3	2.6	0	0.0	1	2.2	4
PIV4	0	0.0	1	0.9	0	0.0	1
PIV total	5	4.4	1	0.9	1	2.2	7
C. pneumoniae	0	0.0	0	0.0	0	0.0	0
M. pneumoniae	6	5.3	3	2.7	2	4.4	1
B. pertussis	9	7.9	0	0.0	0	0.0	9

Abbreviations: *Rhino/Entero* rhinovirus/enterovirus, *RSV* respiratory syncytial virus, *AdV* adenovirus, *hMPV* human metapneumovirus, *Flu B* influenza B virus, *Flu A/unsubtyped, Flu A/ H1, Flu A/H3, Flu A/2009H1* influenza A virus unsubtyped/H1/H3/2009H1, *Cov 229E, Cov HKU1, Cov NL63, Cov OC43* coronavirus 229E/HKU1/NL63/OC43, *PIV 1/2/3/4* parainfluenza 1/2/3/4, *B. pertussis Bordetella pertussis*, *C. pneumoniae Chlamydia pneumoniae, M. pneumoniae Mycoplasma pneumoniae*

Table 3 Prevalence of respiratory pathogens in co-pathogens detected groups

	Rhino/ Entero	RSV	AdV	hMPV	Flu B	Flu A	Cov	PIV	*C. pneumoniae*	*M. pneumoniae*	*B. pertussis*	No.	Prevalence (%, n=45)
	+		+									1	2.2
			+			+						2	4.4
			+					+				1	2.2
			+								+	1	2.2
			+				+					1	2.2
				+			+					1	2.2
						+	+					3	6.7
	+			+								2	4.4
		+		+								1	2.2
	+										+	8	17.8
	+					+						1	2.2
						+		+				3	6.7
		+				+						3	6.7
						+					+	1	2.2
		+						+				6	13.3
								+			+	1	2.2
		+									+	3	6.7
	+							+			+	1	2.2
	+		+							+		1	2.2
	+	+									+	1	2.2
		+				+		+				2	4.4
		+	+			+					+	1	2.2
Total NO.	15	17	8	4	0	16	5	15	0	1	17	45	100.0
Prevalence (%, n = 98)	15.3	17.3	8.2	4.1	0	16.3	5.1	15.3	0	1.0	17.3		

“+” means pathogen detected

Table 4 Antimicrobial prescription rates among patients

	No. (%) of patients with			Total	c^2
	Flu A/B	Non-Flu A/B viral pathogen	Non-pathogen		
Total patients included	97	52	74	223	
Antibiotic prescription	9 (9.3)	15 (28.8)	38 (51.4)	62 (27.8)	37.1
No antibiotic prescription	88 (90.7)	37 (71.2)	36 (48.6)	161 (72.2)	
Anti-influenza prescription	70 (72.2)	6 (11.5)	4 (5.4)	80 (35.9)	98.8
No anti-influenza prescription	27 (27.8)	46 (88.5)	70 (94.6)	143 (64.1)	

Figures

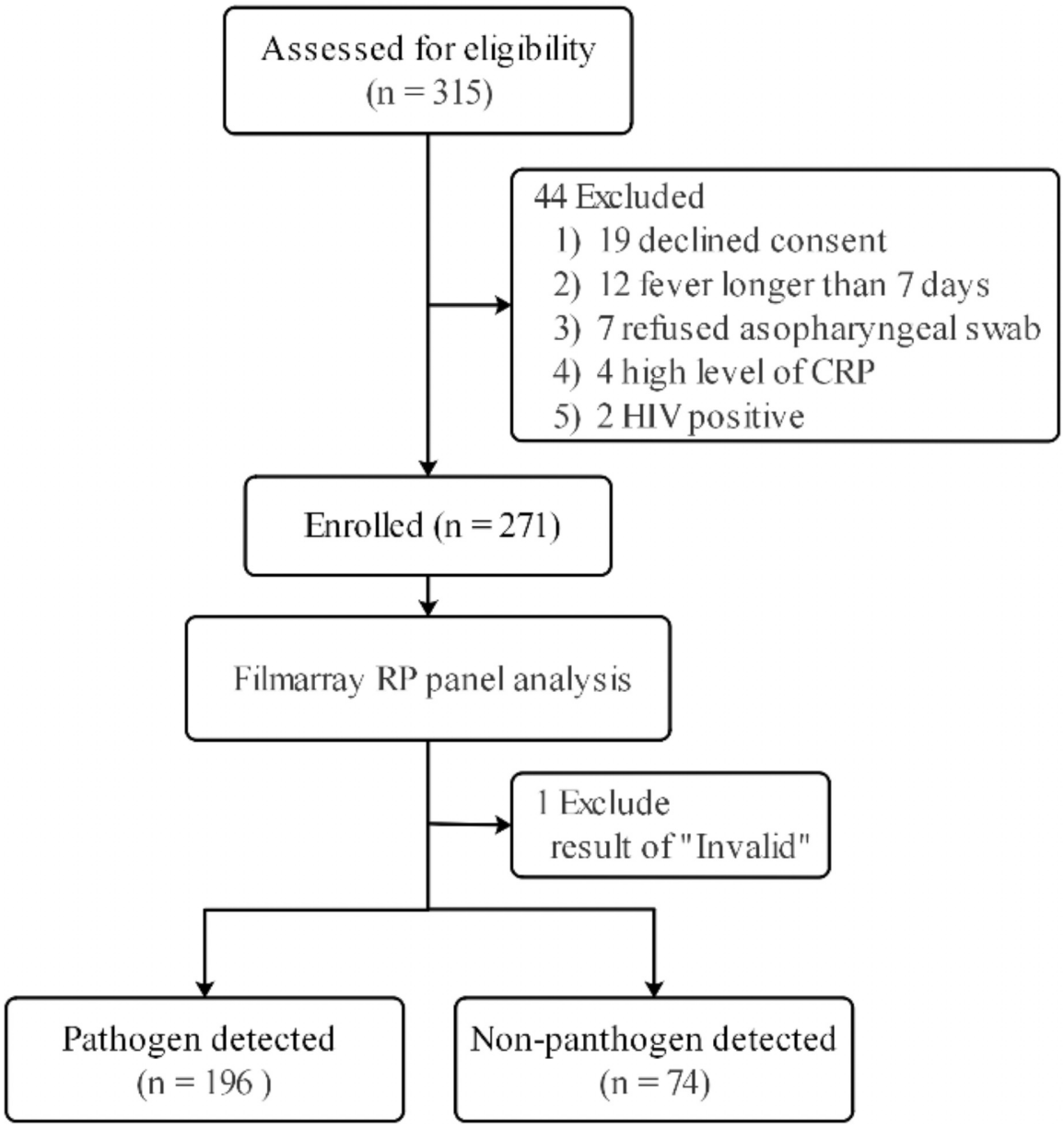

Figure 1

Flow chat of study participants. The result from detection of respiratory pathogens using FilmArray respiratory panel.