The Clinical Application of Filmarray Respiratory Panel in Children with Acute Respiratory Tract Infections in a Pediatric Hospital

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Abstract

Background: Acute respiratory tract infections (ARTIS) are the common diseases in children and common methods frequently fail to identify the infectious etiology especially for viruses. The Filmarray respiratory panel (FARP) can reliably and rapidly identify viruses and bacterial pathogens. This study is to evaluate the performance and clinical significance of FARP in children with ARTIS.

Methods: A total of 90 nasopharyngeal secretion (NPS) samples from children with ARTIS were enrolled. The FARP assay for 17 pathogens and other common methods including direct fluorescence assay (DFA) were performed to analyze these samples. Clinical data of all patients was also collected and evaluated.

Results: Among the 90 samples, 58 samples (64.4%) were positive for 13 pathogens by FARP and 18 positive samples were detected with multiple-virus (31.3%, 18/58). Human rhinovirus/ enterovirus (21.0%%, 17/58) were predominant pathogen, followed by adenovirus (18.5%). Higher proportions of various pathogens were identified in the infant and toddler (0–2 years) groups with human rhinovirus/enterovirus being mostly virus. Adenovirus were common in the group aged 3–5 years, but only three pathogens including *M.pneumoniae*, respiratory syncytial virus, and adenovirus were also found in age group (6-14 years). Among 58 FARP positive patients, significant differences were in antibiotic prescription and use of hormone the single-organism-positive group and the multi-organism-positive group ($P<0.05$). Furthermore, there was significant difference in use of anti-virus and usage of hormone between severe respiratory infections group and non severe respiratory infections group ($P<0.05$).

Conclusions: This study demonstrated that FARP can provide the rapid detection of respiratory virus and atypical bacteria for children, especially with severe respiratory tract infections.

Introduction

Acute respiratory tract infections (ARTIs), represented as major infectious diseases in children with a high morbidity and mortality, are mainly caused by a series of bacteria and virus [1, 2]. Previous estimates found that in 2013, LRIs caused more than 2·6 million deaths worldwide, making them the fifth leading cause of death overall and the leading infectious cause of death in children younger than 5 years [3]. As is known to all, culture and antigen/antibody methods are conventional methods to detect pathogens, but the low sensitivity limit the application in clinical. Introduction of a rapid, sensitive, and specific diagnostic tool is required to understand the epidemiological surveillance and clinical characteristics of RTIs. More recently, advances in polymerase chain reaction (PCR) techniques have aided in the rapid and accurate detection of respiratory pathogens [4–6].

The FilmArray respiratory panel (FARP) is a multiplexed, fully automated nested PCR assay, which can detect seventeen common respiratory virus and three atypical bacterial pathogens with a turnaround time of approximately 1 h [7]. Previous studies have shown that FARP reveals excellent clinical utility over the more traditional laboratory methods of virus culture and direct antigen tests [8–10]. Owing to the

sensitive detection of respiratory viruses, more and more clinical laboratories have introduced this technique to solve intractable cases for clinicians. Data about FARP application in children is still unclear. The goals of the present study are to retrospectively describe the clinical performance of FARP on nasopharyngeal secretion (NPS) samples and also to characterize the clinical effect of FARP in children with severe conditions.

Materials And Methods

Study design

This study was conducted in a children specialized hospital between July 1st, 2017 and June 30th, 2018. Patients from pediatric intensive care unit (PICU) who diagnosed with acute respiratory tract infections were included. This study was approved by the Ethics Committee of Shanghai Children's Hospital. Written informed consent was obtained from the patients' guardians on behalf of the children enrolled in this study.

A total of 90 NPS samples were collected from children under 14 years old. According to the instruction, specimens should be collected on the basis of standard technique and immediately placed in viral transport media (VTM) with minimum sample volume (300 μL). Specimens in VTM should be processed and tested as soon as possible. If storage is required, specimens in VTM can be held at room temperature (18–30 ºC) for up to 4 hours, at refrigerator temperature (2–8 °C) for up to 3 days, or at freezer temperature (<-15 °C) for up to 30 days.

Farp Assay

The FARP assay was performed by multiplex PCR according to the manufacturer's instructions (BioMérieux, France) [11]. In brief, 1 ml of hydration solution provided by the manufacturer was injected into the FilmArray pouch to rehydrate the reagents. Three hundred microliters of NPS was mixed with sample buffer, then injected into the pouch. The loaded pouch was then placed in the FilmArray instrument, and a preprogrammed run was initiated. Results are generated using amplification and melting curve data. The pouch contains all reagents required for specimen extraction, nmPCR (nest multiplex PCR), and results interpretation

The following organism types and subtypes are identified: adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, human metapneumovirus, human rhinovirus/enterovirus, influenza A H1 2009, influenza B, parainfluenza Virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza Virus 4, respiratory syncytial virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. However, human rhinovirus and human enterovirus must be reported as indistinguishable since these they are closely related viruses and cross-positivity between those viruses is possible with the FARP assay [12].

Other Common Methods

The eight viruses included adenovirus, influenza A, influenza B, parainfluenza Virus 1, parainfluenza virus 2, parainfluenza virus 3, respiratory syncytial virus, human metapneumovirus were commonly detected by Direct fluorescence assay (DFA) according to the manufacturer's instructions (Diagnostic hybrids, INC, USA). The antibody of *Mycoplasma pneumoniae* were analyzed by passive particle agglutination and *Bordetella pertussis* were analyzed by culture methods. Other pathogens were not identified in our clinical laboratory.

Statistics Analysis

All statistical analysis was performed by using SPSS 19.0 for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). Clinical testing and the FARP assay were compared using the exact two-sided McNemar's test. A value of $P \leq 0.05$ was considered statistically significant.

Results

Clinical characteristic of enrolled patients

A total of ninety samples were collected from ninety patients in this study. The general characteristics of these patients are presented in Table 1. The average age of all children was 2.55 ± 2.93 years, with 52 male and 38 female children. The children aged between 0 and 14 years were divided into four groups including infants (0–1 year, 34.4%), toddlers (1–2 years, 32.2%), preschoolers (3–5 years, 24.4%) and children (6–14 years, 8.9%). 48 children (53.3%) were diagnosed with severe respiratory infections, and 46.7% of them were observed with underlying diseases including sepsis, heart disease, and intestinal diseases. Furthermore, a majority of children were improved after the treatment during hospitalization and 3 children were died.

Table 1
Clinical characteristics of 90 children enrolled in this study

CharacCstics	All patients (%)	Severe respiratory infections group (%)	Non severe respiratory infection group (%)
No.	90	48	42
Gender			
Male	52 (57.80)	28 (58.3)	25 (59.52)
Female	38 (42.20)	20 (41.7)	17 (40.48)
Age			
< 1 year	31 (34.44)	15 (31.25)	16 (38.10)
1–2 years	29 (32.22)	18 (37.50)	11 (26.19)
3–5 years	22 (24.44)	13 (27.08)	9 (21.43)
6–14 years	8 (8.89)	2 (4.17)	6 (14.29)
Hospitalization stay (days)	18.36 ± 19.31	19.40 ± 20.38	17.17 ± 18.19
Cost (RMB)	61222.26 ± 87707.67	73978.42 ± 101649.49	46643.81 ± 66676.26
Use of anti-virus (%)	20 (22.22)	17 (35.42)	3 (7.14)
Days of antibiotic use (d)	8.68 ± 5.59	8.76 ± 6.25	8.58 ± 4.67
Use of hormone (%)	60 (66.67)	40 (83.33)	20 (47.62)
Underlying diseases			
None	48 (53.33)	24 (50.00)	24 (57.14)
Sepsis	14 (15.56)	10 (20.83)	4 (9.52)
Heart diseases	9 (10.00)	4 (8.33)	5 (11.90)
Intestinal diseases	6 (6.67)	4 (8.33)	2 (4.76)
Central Nervous diseases	5 (5.56)	1 (2.08)	4 (9.52)
Other diseases	8 (8.89)	5 (10.42)	3 (7.14)
Clinical outcome			
Improved	74 (82.22)	37 (77.08)	37 (88.10)
Unhealed	13 (14.45)	9 (18.75)	4 (9.52)

CharacCstics	All patients (%)	Severe respiratory infections group (%)	Non severe respiratory infection group (%)
Died	3 (3.33)	2 (4.17)	1 (2.38)

Distribution Of Pathogens By Farp Assay

Among 58 positive samples, 40 (67.0%, 40/58) had a single organism and 18 (31.0%, 18/58) had multiple organisms. Human rhinovirus/enterovirus was the most prevalent organism in 58 positive samples (29.3%, 17/58), followed by adenovirus (25.9, 15/58), parainfluenza virus 3 (19.0%, 11/58), respiratory syncytial virus (19.0%, 11/58). Other pathogens were as follows: *Mycoplasma pneumoniae* (12.1%, 7/58), influenza A H1 2009 (8.6%, 5/58), human metapneumovirus (6.9% ,4/58), influenza B (5.2% ,3/58), *Bordetella pertussis* (5.2%, 3/58), parainfluenza virus 1 (3.4%, 2/58), coronavirus HKU1 (1.7%,1/58), coronavirus NL63 (1.7%, 1/58), parainfluenza virus 4 (1.7%, 1/58) (Table 2). Several differences were detected among age. Higher proportions of various pathogens were identified in the infant and toddler (0–2 years) groups with human rhinovirus/enterovirus being mostly virus. Adenovirus were common in the group aged 3–5 years, but only three pathogens including *M.pneumoniae*, respiratory syncytial virus, and adenovirus were also found in age group (6–14 years).

Table 2
Distribution of all pathogens in children with respiratory infections

	Total	Sigle positive group	Multi positive group	Severe respiratory infections group (48)	Non severe respiratory infection group (42)
Human Rhinovirius/Enterovirus	17	10	7	9	8
Adenovirus	15	7	8	10	5
Parainfluenza Virus 3	11	4	7	6	5
Respiratory Syncytial Virus	11	5	6	6	5
Mycoplasma pneumoniae	7	2	5	5	2
Influenza A H1 2009	5	2	3	4	1
Human Metapneumovirus	4	2	2	2	2
Influenza B	3	2	1	3	0
Bordetella pertussis	3	1	2	0	3
Parainfluenza Virus 1	2	1	1	1	1
Coronavirus HKU1	1	0	1	1	0
Coronavirus NL63	1	0	1	1	0
Parainfluenza Virus 4	1	1	0	1	0
Coronavirus 229E	0	0	0	0	0
Coronavirus OC43	0	0	0	0	0
Parainfluenza Virus 2	0	0	0	0	0
Chlamydophila pneumonia	0	0	0	0	0

The distribution of multi-organism combinations was depicted in Table 3. A total of 18 multi-organism specimens were detected with 13 various combination types. The combination of human rhinovirus/enterovirus plus parainfluenza virus 3 and adenovirus plus respiratory syncytial virus were the most common combination type. Additionally, the majority of multi-organism-positive specimens were observed with adenovirus and human rhinovirus/enterovirus.

Table 3
Distribution of multi-organisms combinations in children with respiratory infections

Organism combination detected	No.
Human Rhinovirius/Enterovirus + Parainfluenza Virus 3	3
Adenovirus + Respiratory Syncytial Virus	3
Adenovirus + Parainfluenza Virus 3	2
Adenovirus + Influenza A H1 2009	1
Parainfluenza Virus 3 + *Mycoplasma pneumoniae*	1
Human Rhinovirius/Enterovirus + *Mycoplasma pneumoniae*	1
Human Rhinovirius/Enteroviru + Human Metapneumovirus	1
Bordetella pertussis + Human Metapneumovirus	1
Bordetella pertussis + Human Rhinovirius/Enterovirus + Parainfluenza Virus 3	1
Adenovirus + Human Rhinovirius/Enterovirus + Respiratory Syncytial Virus	1
Adenovirus + Parainfluenza Virus 1 + Coronavirus HKU1	1
Influenza A H1 2009 + Respiratory Syncytial Virus + Influenza B	1
Influenza A H1 2009 + Respiratory Syncytial Virus + Coronavirus NL63	1

Comparison Of Farp And Other Methods

The FARP assay was significantly more likely to detect a respiratory virus than DFA assay ($P < 0.05$). Among the ninety samples, 58 samples (64.4%) were identified with 13 pathogens by FARP assay, while only 11 (12.2%) samples were detected 5 viral pathogens (adenovirus, influenza A, respiratory syncytial virus, parainfluenza virus 1, and parainfluenza virus 3) by using DFA method. Among these 11 positive samples by DFA assay, 7 out of them were detected with more than 2 viral pathogens by FARP. Furthermore, the samples were observed by FARP method with 1.7 h, which showed lower turnaround time (TAT) than DFA method with 5.2 h. Seven samples detected with *Mycoplasma pneumoniae* by FARP analysis while only one samples were positive with *Mycoplasma pneumoniae* antibody. Then three *Bordetella pertussis* positive samples were

Clinical Significance Of Pathogens By Farp Assay

The detailed clinical significance of 58 FARP positive children was showed in Table 3. Among these organism positive children, 38 children (65.5%) were diagnosed with severe respiratory infections. According to the number of organisms detected, these children were divided into two groups including the

single-organism-positive group and the multi-organism-positive group (Table 4). There was no significant difference in length of hospitalization stay, hospitalization cost, use of anti-virus, rate of secondary infection, and clinical outcome ($P > 0.05$), while significant difference was observed for days of antibiotic use and usage of hormone between these two groups ($P < 0.05$). Furthermore, there was significant difference in use of anti-virus and usage of hormone between severe respiratory infections group ($P < 0.05$) (Table 5).

Table 4
Comparison of clinical significance between multiple organisms and single organism group by FARP assay

Factors		Multiple organism group(40)	Single organism group(18)	T/X^2	Value of P
Hospitalization stay (days)		15.10 ± 14.83	22.78 ± 16.94	1.485	0.228
Cost (RMB)		56453.93 ± 84859.31	66085.87 ± 103706.31	0.007	0.932
Use of anti-virus (%)		11 (27.50)	9 (50.00)	2.782	0.095
Days of antibiotic use (d)		8.05 ± 4.32	10.72 ± 6.98	5.619	0.021
Use of hormone (%)		24 (60.00)	16 (88.90)	4.84	0.028
Secondary infection (%)		17 (42.50)	9 (50.00)	0.282	0.595
Clinical outcome	Improved	33 (82.50)	16 (88.89)	1.425	0.49
	Unhealed	4 (10.00)	2 (11.11)		
	Died	3 (7.50)	0		

Table 5
Comparison of clinical significance in positive samples between severe respiratory infection group and non severe respiratory infection group

Factors		Severe respiratory infections group (38)	Non severe respiratory infections (20)	T/X2	Value of P
Hospitalization stay (days)		18.89 ± 14.87	14.80 ± 17.46	0.892	0.741
Cost (RMB)		72381.71 ± 98676.056	34859.89 ± 67322.420	1.708	0.114
Use of anti-virus (%)		18 (47.37)	2 (10.00)	8.099	0.004
Days of antibiotic use (d)		8.82 ± 5.72	9.00 ± 4.78	0.13	0.764
Use of hormone (%)		32 (84.21)	8 (40.00)	11.966	0.001
Secondary infection (%		19 (50.00)	7 (35.00)	1.192	0.275
Clinical outcome	Improved	30 (78.95)	19 (95.00)		
	Unhealed	6 (15.79)	0	3.559	0.169
	Died	2 (5.26)	1 (5.00)		

Discussion

Over the past decades, RTIs comprise as the most common disease among children under five years of age with the majority in low- and middle-income countries. The etiology of RTIs always contribute to virus and bacterial, including influenza, respiratory syncytial virus, *Bordetella pertussis, Streptococcus pneumoniae*, and *Haemophilus influenzae*[13, 14]. In general, infrequent isolated pathogens were always found in several severe RTIs cases and viruses were considered as the leading cause, including human rhinovirus/enterovirus, adenoviruses, respiratory syncytial virus, and metapneumovirus [15]. This study is similar to the result conducted previously. Respiratory viruses are responsible for bronchiolitis and pneumonia and can also lead to considerable economic burden in the terms of medical visit [16]. In addition, atypical respiratory pathogens involving in *Bordetella pertussiss, M. pneumoniae*, and *C.pneumoniae*, pose as the emerging respiratory pathogens and have become a health public problem in many countries. Several studies depict that clinical symptoms of atypical respiratory infections is indistinguishable from viral respiratory infections and that co-infection with other virus also exists [17].

Recently, there has been an increasing interest that simultaneous infection with multiple pathogens is increasingly recognized as both common and important for disease manifestation. This study described that 18 children had more than two organisms, with human rhinovirus/enterovirus plus parainfluenza virus 3 and adenovirus plus respiratory syncytial virus being most. It may make the treatment of simultaneous infection more difficult. Then co-infections between virus and bacterial isolates have been

also detected in pediatric patients with ARTIs [18] and this phenomenon was observed in 4 samples with *B.pertussis* or *M.pneumoniae plus virus.*

However, in regard to viruses and atypical organisms, it is truly difficult to detect by traditional culture methods, owing to the long culture period and low sensitivity of these methods. FARP assay, a new technology, has been provided for detecting unidentified pathogens in respiratory samples. Previous studies demonstrated that FARP, which can simultaneously identify 14 viruses and 3 atypical organisms, showed high sensitivity and specificity than other route method introduced in clinical [19, 20]. Our study demonstrated that in children with severe respiratory infections, the FARP assay has a higher positive detection rate than DFA assay available in our laboratory (64.4% vs 12.2%). Additional pathogens including coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, human rhinovirus/enterovirus, parainfluenza Virus 4, *B.pertussis*, *C.pneumoniae*, and *M.pneumoniae* were detected by FARP assay could not identified by DFA method.

Moreover, it is proposed that early diagnosis of pathogens in children with ARTIs could decrease the length of hospitalization stay and reduce the mortality, especially for multiple organism infections. Generally, antibiotics have been commonly prescribed for many children with ARTIs. While the samples were detected with positive pathogen by FARP assay within 1 h, the clinicians should immediately adjust the therapeutic schedule for children. According to the clinical data of these patients, we found that children identified with virus infections received or prolonged antivirus therapy and also reduced the inappropriate use of antibiotics during this process. A previous study reported that the mean duration of antibiotic use was significantly shorter after implementation of FARP than that before the implementation [21]. Furthermore, the length of hospitalization stay and hospitalization cost in the single-organism-positive group were still higher than these in the multi-organism-positive group, although there were no statistical difference in between these two group.

In conclusion, our study revealed that respiratory virus and atypical bacteria are the most frequent pathogens in children, which could not be detected by conventional methods. Comparison of DFA assay, FARP can provide the rapid detection of a wide number of respiratory organisms and especially render a valid choice for urgent pathogens in high-risk patients with severe respiratory infections. However, there still a limitation about the pathogen spectrum of FARP not including all pathogens. Therefore, combination of FARP and other molecular methods can make a significant improvement in diagnostic testing of respiratory pathogens.

Abbreviations

ARTIs: Acute respiratory tract infections; FARP: FilmArray respiratory panel; NPS: nasopharyngeal secretion; PICU: pediatric intensive care unit; PCR: polymerase chain reaction

Declarations

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Authors' contributions

Conceived and designed the experiments: HZ; Performed the experiments: FP, BW, YS, HQ; Analyzed the data: FP; Wrote the manuscript: FP; All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the ethics committee of Shanghai Children's Hospital. Written informed consent was obtained from the patients' guardians on behalf of the children enrolled in this study.

Competing interests

All authors declare that they have no competing interests.

Availability of data and materials

Please contact corresponding athor for data requests.

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