Short Report

Comparison of direct fluorescent test with real-time PCR to detect acute lower respiratory tract virus infections in children from May to July 2014, in a tertiary care hospital in Sri Lanka

HBC Harshani[1], NR Ratnayake[2], CJS Jayamaha[1]

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Abstract

In this study, direct fluorescent test (DFT) and real-time PCR (rtPCR) were compared for the detection of acute lower respiratory tract virus infections caused by respiratory syncytial virus (RSV) A and B, influenza (Flu) A and B, parainfluenza virus (PIV) 1, 2, 3 and 4 and adenovirus (AdV) in children admitted to the Lady Ridgeway Hospital, Colombo.

Nasopharyngeal aspirates/swabs were obtained from May to July, 2014. Cell pellets were subjected to DFT. Extracted viral RNA were subjected to four different real-time multiplex PCR assays to detect the above respiratory viruses.

Of seventy-five specimens, at least one virus was detected in 8 (10.7%) by DFT and in 69 (92%) by rtPCR. The most common viruses detected were RSV-A (5.4%), adenovirus (2.6%) PIV type1/3 (1.3%) by DFT and AdV (70.7%), RSV-A (42.6%), PIV1/3(14.6%), Flu A (8%), and PIV-2/4(1.3%) by rtPCR. Real-time PCR yielded more significant results than DFT in detection of respiratory viruses.

Keywords: Respiratory viruses, Direct Fluorescent test, real-time PCR.

Introduction

In Sri Lanka, acute lower respiratory tract infection (ALRTI) is a leading cause of childhood mortality and morbidity and is responsible for 9% of deaths among children under 5 years of age.[1] Respiratory tract infections are caused by a diverse group of viruses. Bacteria can also cause similar clinical symptoms in acute respiratory tract infections in children.[2] Therefore,

[1]*National Influenza Centre, Department of Virology, Medical Research Institute, Colombo*

[2] *Lady Ridgeway Hospital, Colombo, Sri Lanka*

Address for correspondence: HBC Harshani., Medical Research Institute, Colombo 8, Sri Lanka. Telephone+94771192737. email: hbc.harshani@gmail.com

https://orcid.org/0000-0002-7194-1794

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specific diagnosis based on laboratory investigations is needed for subsequent treatment to provide data for surveillance.

Clinical virology laboratories have historically used direct fluorescent-antibody tests (DFT) and culture to detect viruses causing respiratory tract infections.[3] Though DFT offers a rapid turnaround time, it has many drawbacks, as it is labour intensive, readings could be subjective, and it requires trained technologists.[4]

In real-time PCR, amplification and analysis occur simultaneously and it is appropriate for quantitative analysis.[5] Several studies have shown that the PCR is a highly specific and sensitive method, compared to other tests (DFT and culture) for the diagnosis of acute respiratory virus infections.[6] When the current study was proposed, DFT was the main test used to detect non-influenza viruses in state sector laboratories in Sri Lanka. At that time, though rtPCR was in use, the cost benefit of rtPCR in comparison with DFT had not been evaluated. This study was to determine the in using rtPCR over DFT. The objective of this study was to compare results of DFT and rt-PCR in the detection of RSVA/B, FluA/B, PIV1/2/3/4 and the adenovirus (AdV) in a selected group of children admitted with severe acute respiratory symptoms.

Method

A retrospective, comparison study was conducted from May to July 2014. Nasopharyngeal aspirates(44) and swabs (31) were obtained from children (mean age 3.8 years) admitted to the Lady Ridgeway Hospital, Colombo, with fever, cough, cold or shortness of breath and /or lung crepitations with lower respiratory tract infections. Immunocompromised children and those with a long-term hospital stay were excluded. DFT and rtPCR analyses were done at the Medical Research Institute (MRI), Colombo.

DFT was done using commercial DFT kits (DAKO/Imagen-UK) according to the manufacturer's instructions. Slides with too few respiratory cells were considered inadequate for analysis. Each DFT slide was read twice by two investigators.

Viral RNA extraction on frozen specimens were conducted using the QIAmp RNA mini-kit. Elutes were subjected to four different Altona (GmBH) real-time commercial PCR assays (IVD/CE approved) for detection of the above viruses. SPSS 13.0 software was used for statistical analysis.

Results

Out of 75 specimens, only 8 (10.7%) were positive, 63 (84%) were negative and 4 (5.3%) were inconclusive for at least one virus by DFT, whereas viruses were detected in 92% (n=69) samples by rtPCR. AdV was found in the highest frequency by rtPCR and RSV by DFT. Flu A was not detected by DFT whereas 8% (n=6) was positive by rtPCR. Although one sample with influenza B was detected by DFT, none of the samples were positive for influenza B by rtPCR (Table-1). Fourteen (18.7%) specimens were interpreted as inconclusive by DFT due to insufficient respiratory cells. However, all of them were positive for viruses by rtPCR.

Mixed infections were detected in 34 (45%) samples with 27 (36%) dual infections and seven (9%) as multiple infections by rt-PCR, while there were no mixed infections detected by DFT.

Analytical sensitivity of DFT and rtPCR is 93% and 100% respectively. In this study, the overall detection rates of DFT and rtPCR were 10.6% and 92% respectively (Table 2).

Table 1: Comparison of results of DFT and rtPCR tested for respiratory viruses

Virus	DFT positive		rt-PCR positive	
	n	%	n	%
RSVA	4	5.4(%)	32	42.6
RSVB			7	9.3
PIV type1/3	1	1.3	11	14.6
PIV type 2/4			1	1.3
Influenza A	0	0	6	8.0
Influenza A H1N1			0	0
Influenza B	1	1.3	0	0
Adenovirus	2	2.6	53	70.7

Table 2: Comparison of detection rates, and analytical sensitivity of DFT and PCR

	DFT	rt-PCR
Detection rate by the study	10.6%	92%
Sensitivity by the manufacturer	93%	100%

Discussion

There are very few studies on DFT in Sri Lanka. Athukorale *et al*., (2013)[7] found 10% of influenza A and B positives in children by DFT. Noordeen *et al*., (2014)[8] found 0.42% of influenza A and B in children using an immunochromatographic test. Muthulingam *et al.*, (2014) detected RSV (90%), PIV-2(6%) and influenza (4%) from 98 children under 3 years tested using commercial indirect immunofluorescence and direct immunofluorescence kits.[9]

All specimens in the current study which were positive by DFT were also positive by PCR except one FluB positive specimen. PCR was able to detect at least one virus in all specimens that were interpreted as inconclusive by DFT, probably due to the high sensitivity of PCR.

There were no mixed viral infections found by DFT, whereas 34 specimens were found positive for mixed infections by rtPCR.

Adenovirus accounted for 70.6% of the positive results by rtPCR and was detected in more than 50% of the specimens containing more than one virus. It was also the most frequently detected virus in mixed infections. Parallel results were found by Kuypers *et al*., (2006).[6] A much smaller proportion of the specimens were positive for PIV and AdV by DFT in the same study. However, a study by Jayaweera *et al*., (2021)[10] showed AdV and PIV positive cases in children tested by DFT in the same study period in 2014 but in smaller proportions compared to this study.

According to the results of this study, the real-time PCR assay showed higher detection rates than DFT for the detection of respiratory viruses in clinical specimens from children, except for Flu B. The overall detection rate by DFT (10.6%) is significantly lower ($P < 0.0001$) than the manufacture's sensitivity (92%). However, the rtPCR, detection rate in the study (92%) and the manufacture's sensitivity (100%) were not significantly different. Since we did not compare with a gold standard, we can only present detection rates (not the true sensitivity) in this study.

Conclusions

Real-time PCR yields more positive results than DFT in the detection of respiratory viruses in clinical specimens. PCR can be employed with good results when cells are insufficient in a sample for DFT.

Limitations

The number of samples was low in this study due to the limitation of resources.

Declarations

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