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Original article

A study on pediatric respiratory tract infections in hospitalised children from Chennai

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ABSTRACT

Globally, acute respiratory tract infections (ARTIs) are the leading cause of childhood morbidity and mortality. However, these infections remain poorly understood due to absence of affordable and effective diagnostic tools. In this study, viral acute respiratory tract infections were studied in hospitalised children up to 5 years of age (n = 256) using a commercial multiplex real time PCR. RSV (45.69%%), RV (17.88%), HBoV (7.95%), Influ B (7.28%), HMPV (6.6%), HPIV3 (5.96%) and Influ A virus (3.97%) were the common etiological agents detected. There was no significant correlation between the clinical signs and symptoms in patients with and without a viral aetiology. Multiplexed real time PCR is an important tool for early detection of viral agents of paediatric ARTI.

1. Introduction

Globally, acute respiratory tract infections (ARTI) are the leading causes of mortality and morbidity in children below five years. An estimated 1.9 million childhood ARTI deaths are reported from developing countries, of which 20% occur in India. In India, about 14.3% of infant deaths and 15.9% of deaths in the under-fives are caused by ARTIs. Factors associated with high mortality and morbidity of childhood ARTI include poverty, over-crowding, poor nutrition, poor air quality and misuse of antibiotics.

Influenza A virus (InfluA), influenza A/H1N1pdm09, influenza B virus (InfluB), coronaviruses (NL63, 229E, OC43 and HKU1, SARS CoV-2), human parainfluenza virus types 1 to 4 (HPIV1-4), human metapneumovirus A and B (HMPV), rhinovirus (RV), respiratory syncytial viruses A and B (RSVA/B), adenovirus (HAdV), enterovirus (EV), human parechovirus (HPeV), human bocavirus (HBoV) cause paediatric respiratory disease. HMPV, HPeV and HBoV are emerging respiratory pathogens and their role in pediatric disease is still unclear. RSV remains an important cause of acute bronchiolitis in children under 2 years of age and premature infants particularly those with chronic lung disease or congenital heart disease.[1–5]

The 21st century has witnessed multiple emerging and re-emerging respiratory viruses such as the ongoing SARS CoV-2 pandemic, influenza A/H1N1pdm09, avian influenza (H5N1), severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome coronaviruses {MERS-CoV}.[6,7] Improved surveillance and employment of sensitive detection systems has helped detect and control emerging viruses.

Clinical diagnosis of respiratory tract infections is difficult due to overlap of symptoms caused by different respiratory viral and bacterial pathogens. Bacterial agents of ARTI have largely been controlled by an effective combination of sensitive diagnostic systems, antibiotics and vaccines. Viral ARTIs are the most common reasons for hospitalisation of children in India yet their aetiology and burden in children <5 years remains poorly understood due to the absence of affordable and efficient diagnostic systems[6–8]).

Early and robust detection systems for viral ARTIs in routine diagnostic microbiology laboratories can help avoid unnecessary antibiotic usage and prolonged hospitalisation, especially in moderately severe cases. With early diagnosis, contagious hospitalised patients can be isolated to prevent outbreaks. Real time PCR is currently the gold standard for laboratory diagnosis of ARTIs. It is available in multiplexed formats and detects multiple viruses, up to 4 pathogens, in a single assay. Its high sensitivity and specificity permit increased detection of respiratory viruses in children with ARTI. It is also an excellent tool for surveillance of emerging and re-emerging viruses.[9–11]

Streptococcus pneumoniae (SPN) is a nasopharyngeal coloniser and respiratory pathogen in young children. In India, introduction of 13-valent conjugate pneumococcal vaccine in 2017, for infants and young

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children, has greatly reduced the burden of paediatric pneumococcal pneumonia. Routine surveillance of nasopharyngeal SPN serotypes helps understand circulation of non-vaccine serotypes.[3] The study was conducted to characterize the viral and clinical spectrum of ARTI in children below five years, employing a commercial multiplexed real time PCR. Additionally serotyping of nasopharyngeal pneumococci was undertaken to understand colonising non-vaccine serotypes in an era of pneumococcal vaccine.

2. Materials and methods

2.1. Materials

This study was conducted from September 2019 to February 2020 in children up to five years of age. Children hospitalised at Kanchi Kamakoti Childs Trust Hospital (KKCTH), Chennai with WHO criteria for pneumonia with tachypnea or wheeze with or without hypoxia were included. Those with primary cardiac failure, severe metabolic acidosis without evidence of respiratory tract infection (RTI) were excluded. Informed consent was obtained before enrolment and all relevant clinical data captured prospectively in a case report form (CRF). This study was cleared by the Institutional Ethics Committee (IEC) for its scientific content and ethics (IEC-11/OCT2017-IRB min dt 25.10.2017).

2.2. Methods

Nasopharyngeal (NP) swab was collected from enrolled subjects in viral transport medium (VTM). Total nucleic acids were extracted from NP samples using the Versant 1.0 Reagent kit (Siemens, Belgium). Automated extraction (VERSANT kPCR, Siemens) was done if load was more 10 patient samples.

Multiplex Real time PCR was done with Fast Track Diagnostics (FTD, Fast-Track Diagnostics, Junglinster, Luxembourg) Respiratory Pathogens 21 plus kit which can detect 20 viral and 5 bacterial pathogens including InfluA, influenza A/H1N1pdm09, InfluB, coronaviruses (NL63, 229E, OC43 and HKU1), HPIV1-4), HMPV, RV, RSVA/B, HAdV, EV, HPeV, HBoV and *S. pneumoniae*. . Real time PCR was done on QuantStudio 5 Dx (Thermo Fisher Scientific ®). Testing was done as per the manufacturer's instructions. An internal control (IC) was used to validate the real time PCR assays. A Ct $\leq$ 33 was considered positive for all viral targets.

2.2.1. Streptococcus pneumoniae (SPN) typing

DNA from samples were extracted using Qiagen DNA mini kit as per the manufacturer's instructions. Real time PCR was performed on extracts using *lytA* as target for the detection of *S. pneumoniae*. The *lytA* positive specimens were further serotyped by Triplex real time PCR using the primers and probes as recommended by CDC. (https://www.cdc.gov/streplab/pneumococcus/resources.html). Real time PCR was done on Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument. A Ct Value of $\leq$35 is considered positive for all the targets. Serotyping was performed at the Department of Clinical Microbiology,CMC, Vellore.

3. Results

Overall, 256 children were recruited, of which 170 (66.4%) were males and 86(33.59) females. Age distribution was as follows: $\leq$1 year (n = 148), >1–2 years (n = 56) and >2–5 years (n = 52). . In children $\leq$5 years of age, 188 (73.43%) had laboratory diagnosis of viral RTI (VRTI) mono and co-infections. Of these, 151(58.98%) were mono-infections and 37 (14.45%) were co-infections.

Among the mono-infections, RSV was the most common etiological agent (n = 69, 45.69%) followed by RV (n = 27, 17.88%), HBoV (n = 12, 7.95%) InfluB (n = 12, 8%), HMPV (n = 10, 6.6%), HPIV3 (n = 9, 5.96 %) and InfluA (n = 6, 3.97%). There were few HAdV (n = 2) and influenza A/H1N1pdm09 (n = 4) mono-infections. The viruses involved in co-infections were RSV (n = 23), RV (n = 16), HPeV (n = 10), HAdV (n = 8), HPIV3 (n = 6), HBoV (n = 5), InfluB and HMPV (n = 3 each). (Tables 1 and 2).

Children with evidence of viral infection presented with fever of upto a week's duration (1–7 days). All the subjects had an axillary temperature of $\geq$100.4 °C, were tachypneic and presented with one or more danger signs including severe respiratory distress, inability to feed/drink, incessant vomiting, lethargy, convulsions or stridor in a calm child. Overall, all presented with respiratory distress warranting medical care. The final diagnosis of patients with viral RTI included pneumonia, bronchiolitis and wheeze associated lower respiratory infection (WALRI). All patients had a favourable outcome and there were no deaths.

RSV mono-infections were common amongst those up to 1 year of age (n = 55, 79.71%). Most of them occurred during the monsoon months of September, October and November (n = 65, 94%). Average Ct value was 23.72. Oxygen saturation levels in these patients ranged from 88% to 99% with seven requiring supplemental oxygen. The final diagnosis included bronchiolitis, wheeze associated (WALRI) and pneumonia.

RV mono-infections were common amongst those up to 1 year of age (n = 16, 59.25%). Majority of these infections occurred during November, December, January and February. Average Ct value was 26.74. One subject had evidence of central cyanosis with oxygen saturation of 68%. HPIV3 mono infections occurred during December, January and February. All except one, were $\leq$2 years of age.

In this study, there was no significant difference between the clinical severity of RTI caused by co-infections and mono-infections. One patient diagnosed as community acquired pneumonia caused by HBoV and HMPV co-infection also had a high nasopharyngeal pneumococcal load (Ct,24).

HPeV was detected in 10 patients below 2 years of age. All were co-infected with RSV The mean number of days of hospitalisation was 3.5. Ct values of HPeV were low (22.75) indicating high concentrations of viral RNA. The Ct of HPeV RNA closely mirrored that of RSV, indicating high viral loads of both.

Overall, in children $\leq$1 year, three fourths of the RTIs were RSV followed by RV, HBoV and HPIV3. In those between >1 and 2 years, RSV was the most common followed by Influenza B while in those >2 years, RV and Influenza A/B virus infections were the most common respiratory pathogens.

3.1. Nasopharyngeal colonisation by SPN and viral ARTI

Overall, of 256 subjects, only 233 were tested for SPN. Of these 84 (33%, n = 84) had evidence of nasopharyngeal SPN colonisation. 44 (52.38%) subjects diagnosed with nasopharyngeal pneumococcal carriage had received PCV vaccination. SPN colonisation was seen in $\leq$1

Table 1
Mono-infections and co-infections.

Virus	Mono infection	Co-infections
Influ A	6	1
Influ B	12	3
H1N1	4	1
RV	27	16
HPIV2	0	0
HPIV3	9	6
HPIV4	0	1
HPIV1	0	1
HBoV	12	5
HMPV	10	3
RSV A/B	69	23
HPeV	0	10
HAdV	2	8
Total	**151**	–

Table 2
Details of co-infections with respiratory viruses.

Viruses	Number of patients
HPIV1+HBoV + HMPV	1
HAdV + RV	3
HBoV + HMPV	2
HPIV3+HBoV	1
HPIV3+RV	3
HPIV3+HAdV	1
HPeV + RSV	9
HPIV3+HAdV + RV	1
RSV + HBoV	1
RSV + RV	6
RSV + Influ B	2
RSV + H1N1+Influ A	1
RSV + HAdV	2
RSV + HAdV + Influ B	1
RV + EV	2
HPeV + RSV + RV	1
Total	**37**

year of age (n = 39), >1 to 2 years (n = 23) and >2 years (n = 22). A subgroup of SPN positives (n = 39) were serotyped based on availability of sample and Ct values ≤ 35. In this study, vaccine serotypes, 6B, 7F, 9V, 14, 19F, 23F were detected along with non-vaccine serotypes, 16F, 15A/F, 11A/D. Of the 24 subjects with sero-typable SPN colonisers, 18 (75%) were vaccinated and ≤2 years. Of 15 subjects with nasopharyngeal non-typable SPN colonisers, 10 (67%) were vaccinated and ≤2 years of age (Table 3).

4. Discussion

In India, acute RTI accounts for 69% of all communicable diseases and severe acute respiratory infection (SARI) is one of the leading causes of mortality in the under-five. Studies on pediatric RTI in India, focusing on prevalence, surveillance, disease burden, diagnostics of viral respiratory illnesses are few and far between.

This study explores the clinical and viral spectrum of RTI in the under-five in a tertiary care pediatric facility in Chennai. The study was undertaken from September 2019 to February 2020. There was a statistically significant difference between the number of males versus females enrolled in the study ($p < 0.05$), similar to a previous study.[9]

Our findings indicate that in Chennai, RSV and RV are the most common viral cause of RTI in children up to five years. Other respiratory pathogens detected were HBoV, InfluB, HMPV, HPIV3, InfluA and few cases of HAdV and influenza A/H1N1pdm09 mono-infections in order of their occurrence. A study from a community-based cohort of rural children in north India, <10 years, reported RSV as the most commonly detected pathogen followed by parainfluenza virus (PIV), HMPV and influenza viruses {IV}.[1]

A report form Bangalore on hospitalised children <5 years with a diagnosis of RTI had RSV as the most detected pathogen.[7] In a study conducted in Rajasthan from 2012 to 2013, HMPV was the common cause of SARI in children up to 5 years.[8] In eastern India, RSV and Flu B are reportedly the predominant pathogens causing RTI in children <5 years.[9]

RSV has a greater prevalence in India compared to other respiratory pathogens. Currently, RV infections are increasingly being reported with the availability of sensitive detection systems. Worldwide, RV is the most prevalent respiratory pathogen causing RTI, followed by RSV. Compared to the rest of the world, in India, prevalence of HMPV and InfluB is higher while that of InfluA, lower.

This study employs Fast Track Diagnostics (FTD) (Fast-Track Diagnostics, Junglinster, Luxembourg) Respiratory Pathogens 21 plus kit which can diagnose 20 viral and 5 bacterial respiratory pathogens. The test is done sequentially in six RT-PCR real time assays targeting different pathogens sequentially. Each assay can detect up to 4 targets.

Table 3
SPN serotypes.

Serotypes	P
Serotype 9V/A	5
Serotype 15A/F	3
Serotype 16F, Serotype 11A/D, Serotype 18C/B/A/F	3 (1 each)
Serotype 23F	3
Serotype 19F	2
Negative for all 21 serotypes	15
Serotype 6A/B/C/D	7
Serotype18 C/B/A/F, Serotype 19F, Serotype 23F (Mixed)	1
Total	**39**

The kit is expensive and includes respiratory pathogens not relevant to India. However,the multiplex panels used are not in concurrence with pediatric data from India.[12,13] In light of this study, in-house multiplex Real Time PCR can be designed and custom-made to provide reliable and affordable viral diagnostics with optimal turnaround time (TAT). Multiplexing viral targets based on patients' age permits optimal use of scarce resources. Our results indicate that for children up to 5 years of age, first-level multiplexing of RSV, RV, InfluB and HBoV followed by a panel of SARS-CoV-2, HPIV3, InfluA and HMPV would improve turnaround time (TAT) and save costs. However larger studies would be needed to provide more data on design of multiplexing panels for diagnosing pediatric RTI in India.

HBoV has been detected in symptomatic and asymptomatic children with higher rates of detection in children up to 1 year of age. Bharaj et al., in 2010, tested nasopharyngeal aspirates from children <5 years of age with acute respiratory tract infection and concluded that HBoV causes upper and lower respiratory tract disease in young children in India. HMPV infections have been reported from India; mean age of hMPV infected hospitalised children with ARTI was 9.1 ± 7.1 months. HMPV and HBoV can be considered important causes of wheezing; this association was significantly reflected in our study with nearly all the patients reporting wheezing as a clinical symptom. However, unlike RSV, HMPV and HBoV infections are milder and rarely result in mortality. Our study indicates a low level of circulation of HPeV; an "ambiguous" cause of paediatric ARTI. We suspect that coinfection of HPeV with other respiratory viruses may confer a higher chance of low respiratory tract involvement. acute bronchiolitis or pneumonia. This study can draw the following conclusions: there is a low frequency of HPeV circulation in children with LRTI, HPeV may co-circulate only with other respiratory viruses to cause seasonal ARTI particularly in the rainy months of the year or they may be innocent bystander viruses.[14,15]

Higher pneumococcal colonisation density has been linked to respiratory virus co-infections. PCV was introduced into the Universal Immunization Programme (UIP) in 2017. In this study, vaccine serotypes, 6B, 7F, 9V, 14, 19F, 23F were detected along with non-vaccine serotypes, 16F, 15A/F, 11A/D. As PCV vaccination gains ground, routine surveillance of SPN colonisers is important.

The study explores the impact of multiplexed real time PCR in the diagnosis of paediatric viral RTI. A limitation of the study was its short duration (September 2019 to February 2020) due to emergence of the ongoing COVID-19 pandemic. In-house multiplexing is cost effective, helps diagnose a spectrum of clinically relevant viruses in a reasonable TAT, can be operationally simple if standardized well: all this without compromising quality.

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Declaration of competing interest

None.

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