ORIGINAL ARTICLE

RAPID DETECTION OF RESPIRATORY PATHOGENS USING A MULTIPLEX PCR ASSAY AMONG HOSPITALIZED CHILDREN WITH ACUTE RESPIRATORY INFECTION

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Background: Acute respiratory infection (ARI) is a major cause of morbidity and mortality among children worldwide however, local data on the etiologic diagnosis of ARI are limited.

Objectives: To determine the prevalence and the most commonly detected respiratory pathogens using a multiplex PCR assay, known as the Respiratory Panel, among hospitalized children with ARI and compare their clinical and laboratory differences.

Methods: This is a cross sectional study of children with ARI who were tested with a multiplex PCR assay. Retrospective chart review was done on these patients admitted from January 2018 to February 2020.

Results: There were 47 charts reviewed, mean age was 4.2 years old. Out of 47 patients, 36 (76.6%) tested positive for a pathogen. Respiratory syncytial virus (RSV) being the most common followed by Influenza A/H1-2009 and Human metapneumovirus (hMPV). Two patients had viral co-infections and no bacteria were detected on all subjects. 61.7% patients were started on antibiotics on admission. Fever and cough were the most common sign and symptom, respectively. Normal WBC (68% with neutrophilic predominance) and platelet were detected in 72.3% and 70.2% of patients, respectively; 50% of patients had normal CRP and 60.5% had abnormal findings on chest x-ray. Only the presence of chest x-ray findings was found to have a higher probability of yielding a positive Respiratory Panel p=0.27.

Conclusion: Among admitted patients with ARI, 76.6% tested positive for a respiratory pathogen. All were caused by viruses presenting as nonspecific manifestations - fever and cough. Clinical manifestations, CBC and CRP showed no association with the Respiratory Panel result while abnormal chest x-ray had a higher probability of yielding a positive Respiratory Panel result.

KEYWORDS: *Acute respiratory infection, Respiratory Panel, Multiplex PCR assay*

INTRODUCTION

Bacteria and viruses have been reported as the main causes of acute respiratory infections (ARIs). An estimated 50% of all illnesses in all age groups, and approximately 75% of illnesses in young children are viral upper respiratory tract infections (URTI).[1] In children under 5 years, ARIs are mainly due to viruses -- RSV, parainfluenza viruses, influenza virus A and B, and hMPVs are the most common viruses isolated.[2] In most developing countries like the Philippines, data on the etiologic diagnosis are limited, primarily because of difficulties in obtaining adequate samples and the low sensitivity of presently available diagnostic methods, such as blood culture and serological tests. Viral etiology studies likewise are uncommon.[3]

A community-based ARI research named Philippine Control of Acute Respiratory Infections program (Phil-CARI) was established in 1981. By 1989, the community-based research collaboration between the Research Institute for Tropical Medicine (RITM) and the Australian government generated about 7 years' worth of valuable local data on baseline ARI incidence and risk factors. They confirmed the high incidence of ARI, at 6.1 episodes per child-year, among Filipino children less than 5 years old in depressed urban communities in Metro Manila, with a peak age incidence between 6 and 23 months. The incidence of acute lower respiratory tract infection (LRTI) was also found to be exceedingly high, at 0.5 episode per child-year.[4] In 1984, an outpatient clinic and hospital study was conducted among Filipino children 5 years old and below living in a periurban slum and a middle-class neighborhood. A total of 198 viral infections was confirmed in 162 patients (51.9%), 42.3% with single viral infection and 9.6% with mixed (two or more) infection. The infections were measles (21.4%), influenza A (15.9%), parainfluenza types 1, 2, and 3 (8.8%), RSV (7.1%), influenza B (5.8%), enteroviruses (5.1%), adenoviruses (39%), herpes simplex virus (1.6%), and cytomegalovirus (1.3%).[5] From April 1990 to December 1992, another local study on the etiology of acute LRTI was identified in 119 (36.9%) of 317 hospitalized children below 5 years of age. A higher proportion of respiratory viruses (27.2%) than bacterial agents (10.7%) were identified through blood culture, nasopharyngeal aspirate culture and immunofluorescence technique. Viral agents (adenovirus, RSV, parainfluenza 3, influenza A and influenza B) and bacterial agents (mainly *Haemophilus influenzae* and *Streptococcus pneumoniae*) are the pathogenic agents involved in acute LRTI among Filipino children less than 5 years old.[3]

After the discovery of polymerase chain reaction (PCR), there are many important milestones in the evolution of diagnostic molecular tests. A multiplex point-of-care diagnostic technology (MPOCT) can test the presence of multiple infectious pathogens within a specimen. The test results can be obtained within 15 minutes to several hours. The development of new molecular panel diagnostics that can provide results this fast would provide both clinical and economic benefits. Analysis of the results provides the clinician with an opportunity to administer directed therapies in a short time. The set of tests on a multiplex technology is known as a test panel. Syndromic test panels are designed to test for multiple diseases associated with a similar set of symptoms, or a syndrome and these panels help in the evaluation of the etiology of the disease at the point of care. Respiratory panels and gastrointestinal panels are two examples of syndromic panels.[5] MPOCT has been utilized in other countries and is now being used as rapid diagnostic tests in many parts of the world. St. Luke's Medical Center, Quezon City, a tertiary private hospital, acquired a Biofire® FilmArray® Multiplex PCR system (mPCR) last January 2018. This mPCR system, commonly known as the Respiratory Panel, provides a rapid and accurate identification of causative agents of respiratory tract infections. Thus, we would like to determine the prevalence of the respiratory pathogens and to determine the most common pathogens involved among admitted children with ARI. We would also like to determine the correlation of the presence and absence of a respiratory pathogen with the clinical manifestations, laboratory and chest x-ray findings of admitted children with ARI.

MATERIALS AND METHODS

Study design and participants

This is a cross-sectional study, using retrospective chart review of pediatric patients admitted in St. Luke's Medical Center – Quezon City who were tested with Biofire® Filmarray® Respiratory Panel for respiratory pathogens via mPCR from January 2018 to February 2020.

Inclusion and exclusion criteria for subject selection

This study enrolled consecutive pediatric patients (below 19 years old) admitted for ARI and underwent Biofire® Filmarray® Respiratory Panel (with positive or negative results). An ARI is defined as the presence of any symptom and/or signs such as cough, difficulty of breathing, sore throat or rhinorrhea. Patients with chronic medical conditions as defined by ICD-9 code were excluded from the study.[6]

Description of the study procedure

Between January 2018 to February 2020, records of pediatric patients with acute respiratory infection who were tested with Biofire® Filmarray® Respiratory Panel were included. The Respiratory Panel is a multiplex PCR system that tests for the presence of Adenovirus (AdV), Coronavirus HKU1, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, hMPV, Human RhV/ Enterovirus, Influenza A, Influenza A/H1, Influenza A/H1-2009, Influenza A/H3, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, RSV, *Bordetella pertussis, Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. The test is manufactured in Salt Lake City, Utah. Sensitivity ranges from 94.6-100% for most of the viruses, with the lowest sensitivity of 90% for influenza A. Specificity is 98.3-100% for most of the viruses in the test with 94.6% for hMPV and 89.1% sensitivity for RSV. A nasopharyngeal swab is obtained and placed on a Filmarray® reagent pouch. This pouch stores all the necessary reagents for sample preparation, reverse transcription, PCR and detection then placed on the Filmarray® machine. The Filmarray® software automatically generates a result for each target in a single report within an hour. A Respiratory Panel test roughly costs 23,000 in Philippine pesos.

Characteristics and variables such as age, sex, clinical presentations, CBC, CRP, chest x-ray results and management on admission were determined in this study. The prevalence of respiratory pathogens, the most commonly detected pathogens and the signs and symptoms associated with either Respiratory Panel positive or negative were analyzed.

Sample size estimation

Sample size was calculated based on the population proportion estimation. Sample size as calculated based on the proportion of patients positive for RSV (it having the highest sample size calculation possible of all respiratory microorganisms). Assuming that the proportion of patients with RSV is 48% with a maximum allowable error of 7.5-10%, and a reliability of 80%, sample size required is 42-74.[7]

Mode of data analysis

Determination of Respiratory Panel result, prevalence and identification of respiratory pathogens, signs and symptoms of ARI, laboratory and chest x-ray findings were determined using frequency and percentage. Association of the clinical characteristics and laboratory and chest x-ray findings with the Respiratory Panel result was determined using Fisher's exact test. Level of significance was set at $p<0.05$.

Ethical considerations

The study upholds the highest ethical standard of confidentiality, transparency and integrity in processing personal information. The study abides by the Principles of the Declaration of Helsinki (2013) and was conducted along the Guidelines of the International Conference on Harmonization-Good Clinical Practice (ICH-GCP). The clinical protocol and all relevant documents were reviewed and approved by the SLMC Institutional Ethics Review Committee on October 14, 2019. Patient confidentiality was respected by ensuring anonymity of patient records. Each patient document is coded and did not contain any identifying information in order to ensure confidentiality. The chart review was done by the author and was done in the hospital premises. All study data were recorded and investigators were responsible for the integrity of the data.

The manner of disseminating and communicating the study results guarantee the protection of the confidentiality of patient's data. All study-related documents such as the all versions of the protocol, ethical clearance, data collection forms, hard copies of source documents, are kept and stored by the principal investigator in strict confidentiality; after which they will be shredded. Data collection commenced upon approval of the research protocol by the Institutional Review Board and Institutional Ethics Review Committee. The study was self-funded, one of the authors though was a lecturer for Biofire® Filmarray® Respiratory Panel in 2020. The authors deny any other conflicts of interest.

RESULTS

Overall, 65 Respiratory Panel tests were done in children within the study period. All tested patients presented with signs and symptoms of ARI but 5 were done as outpatient. 13 patient records were found to have chronic illnesses like SLE, severe pneumonia and septic shock with multi-organ dysfunction syndrome, acute flaccid myelitis, SMA type 1, Down's syndrome and acute lymphoblastic leukemia, chronic lung disease and were excluded in the study while 1 patient had 4 Respiratory Panel tests done within the study period. A total of 47 patient records met the inclusion and exclusion criteria.

Table 1 shows the characteristics of admitted patients with ARIs included in this study. Majority of the patients were females comprising 53.2% and most of which belong to the age group of 1-5 years old. On admission, 61.7% were started on antibiotics. Among those who tested positive for Respiratory panel, 58.3% were started on antibiotic. After the positive Respiratory Panel result was relayed, 23.8% discontinued the antibiotic. Among 11 patients who tested negative for Respiratory Panel, 72.7% were started on antibiotic. After the negative Respiratory Panel result was relayed, 14.3% discontinued the antibiotic.

Table 1: Demographic Profile of patients with ARI who underwent Respiratory Panel

		***n* = 47(%)**
Gender	Male	22 (46.8)
	Female	25 (53.2)
Age (in years)	<1	8 (17)
	1-5	26 (55.3)
	6-12	10 (21.3)
	13-18	3 (6.4)
Management on admission	Supportive	18 (38.3)
	Antibiotic	29 (61.7)

At least 1 pathogen was detected on 76.6% of patients, all of which were viruses. Single viral infection was detected in 34 (72.3%) and viral co-infections in 2 (4.3%).

All respiratory pathogens detected were viruses, RSV being the most prevalent followed by influenza A/H1-2009 and hMPV. Co-infection of viruses were detected in two patients: RSV-RhV/enterovirus and coronavirus 229E-hMPV. No viral-bacterial and bacterial co-infections were detected.

Table 2: Respiratory pathogens in children with ARI who underwent Respiratory Panel

Pathogen	***n* = 47(%)**
None detected	11 (23.4)
RSV	10 (21.3)
Influenza A/H1-2009	8 (17)
hMPV	5 (10.6)
Influenza B	3 (6.4)
Human RhV/Enterovirus	3 (6.4)
Parainfluenza Virus 4	2 (4.3)
Influenza A/H3	2 (4.3)
Parainfluenza Virus 2	1 (2.1)
Influenza A/H1	1 (2.1)
Coronavirus NL63	1 (2.1)
Coronavirus 229E	1 (2.1)
Adenovirus	1 (2.1)
Parainfluenza Virus 1	0
Parainfluenza Virus 3	0
Influenza A	0
Coronavirus OC43	0
Coronavirus HKU1	0
Bordetella pertussis	0
Chlamydophila pneumoniae	0
Mycoplasma pneumoniae	0

In children 5 years old and below, ARIs were caused mainly by RSV (21.3%, n = 10), Influenza A/H1-2009 (12.8%, n = 6) and hMPV (10.6%, n = 5). All children positive for RSV were noted to be less than 5 years old (see Table 3).

Table 3: Respiratory pathogens per age group in children with ARI

Pathogen	**Age (in years), *n*= 47 (%)**			
	<1	**1-5**	**6-12**	**13-18**
None detected	1 (2.1)	5 (10.6)	3 (6.4)	2 (4.3)
RSV	6 (12.8)	4 (8.5)	0	0
Influenza A/H1-2009	0	6 (12.8)	1 (2.1)	1 (2.1)
Human Metapneumovirus	0	5 (10.6)	0	0
Influenza B	0	1 (2.1)	2 (4.3)	0
Human Rhinovirus/ Enterovirus	1 (2.1)	1 (2.1)	1 (2.1)	0
Parainfluenza Virus 4	0	1 (2.1)	1 (2.1)	0
Influenza A/H3	0	2 (4.3)	0	0
Parainfluenza Virus 2	0	0	1(2.1)	0
Influenza A/H1	1(2.1)	0	0	0
Coronavirus NL63	0	0	1(2.1)	0
Coronavirus 229E	0	0	1(2.1)	0
Adenovirus	0	1 (2.1)	0	0
TOTAL	**9 (19.2)**	**26 (55.3)**	**11 (23.4)**	**3 (6.4)**

Table 4 shows that the most common physical examination finding was fever having a mean maximum temperature of 39.2°C, followed by retractions and nasal obstruction or discharge. Cough was the most common symptom followed by rhinorrhea. When positive and negative Respiratory Panel results were compared, a respiratory pathogen is more likely to be detected with the presence of fever, retractions, tachypnea, wheeze, crackles, cough and difficulty of breathing (see Table 5). No statistically significant relationship was noted on all variables measured.

Table 4: Clinical manifestations of children with ARI who underwent Respiratory Panel

Signs	*n =47* (%)
Fever	43 (91.5)
Nasal obstruction/discharge	36 (76.6)
Retractions	37 (78.7)
Tachypnea	15 (31.9)
Wheeze	18 (38.3)
Crackles	23 (48.9)
Symptoms	
Rhinorrhea	37 (78.7)
Sore throat	5 (10.6)
Cough	42 (89.4)
Difficulty of breathing	14 (29.8)

Table 5: Comparison of signs and symptoms of ARI between positive and negative Respiratory Panel

Variable		**Positive (*n*=36) *n* (%)**	**Negative (*n*=11) *n* (%)**	***p-value***
SIGNS				
Fever	Present	34 (79.1)	9 (20.9)	0.229
	Absent	2 (50)	2 (50)	
Nasal obstruction/ discharge	Present	27 (75)	9 (25)	0.492
	Absent	9 (81.8)	2 (18.2)	
Retractions	Present	16 (88.9)	2 (11.1)	0.111
	Absent	20 (69)	9 (31)	
Tachypnea	Present	12 (80)	3 (20)	0.507
	Absent	24 (75)	8 (25)	
Wheezes	Present	15 (83.3)	3 (16.7)	0.312
	Absent	21 (72.4)	8 (27.6)	
Crackles	Present	20 (87)	3 (13)	0.101
	Absent	16 (66.7)	8 (33.3)	
SYMPTOMS				
Rhinorrhea	Present	28 (75.7)	9 (24.3)	0.570
	Absent	8 (80)	2 (20)	
Sore throat	Present	2 (40)	3 (60)	0.076
	Absent	34 (81)	8 (19)	
Cough	Present	33 (78.6)	9 (21.4)	0.332
	Absent	3 (60)	2 (40)	
Difficulty of breathing	Present	12 (85.7)	2 (14.3)	0.287
	Absent	24 (72.7)	9 (27.3)	

Table 6 summarizes the laboratory and radiologic findings gathered. Leukopenia and thrombocytopenia were detected in 14.9% and 4.3% of patients, respectively. Mean WBC was 10.08 x 10^9/L and stabs were seen on 12.8% of patients and 68% had neutrophilic predominance. Mean platelet count was 307 x 10^9/L. CRP was done on 24 patients, half of patients had abnormal CRP, 20.8% had equivocal CRP and 29.2% had high CRP. Chest x-ray was done on 38 patients, 60.5% were radiologically diagnosed with LRTI. On Table 7, positive and negative Respiratory Panel results were compared and the presence of x-ray findings had a higher probability of yielding a positive Respiratory Panel (p = 0.027).

Table 6: Laboratory and radiologic findings of children with ARI who underwent Respiratory Panel

Laboratory findings	***n* (%)**
WBC	*n=47*
Normal	34 (72.3)
Low	7 (14.9)
High	6 (12.8)
Predominance of differential count	*n=47*
Neutrophilic	27 (57.4)
Neutrophilic + stabs	5 (10.6)
Lymphocytic	14 (29.8)
Lymphocytic + stabs	1 (2.1)
Platelet	*n=47*
Normal	33 (70.2)
Low	2 (4.3)
High	12 (25.5)
CRP	*n=24*
Normal	12 (50)
Equivocal	5 (20.8)
High	7 (29.2)
Findings on chest x-ray	*n=38*
With radiographic findings	23 (60.5)
Normal	15 (39.5)
X-ray findings	*n=24**
Streaky densities	7 (29.2)
Linear/Hazy opacities	7 (29.2)
Interstitial lung findings	7 (29.2)
Consolidation	2 (8.4)
Increased peribronchial markings	1 (4.2)

*One patient had more than 1 CXR finding.

Table 7: Comparison of laboratory and chest x-ray findings between positive and negative Respiratory Panel

Variable		**Positive (*n*=36) *n* (%)**	**Negative (*n*=11) *n* (%)**	***p-value***
WBC	Normal	25 (73.5)	9 (26.5)	0.266
	Low	7 (100)	0 (0)	
	High	4 (66.7)	2 (33.3)	
Predominance of differential count	Neutrophilic	20 (74.1)	7 25.9)	0.927
	Neutrophilic + Stabs	4 (80)	1 (20)	
	Lymphocytic	11 (78.6)	3 (21.4)	
	Lymphocytic + Stabs	1 (100)	0 (0)	
Platelet	Normal	26 (78.8)	7 (21.2)	0.507
	Low	2 (100)	0 (0)	
	High	8 (66.7)	4 (33.3)	
CRP	Results	N=19	N=5	0.826
	Normal	10 (83.3)	2 (16.7)	
	Equivocal	4 (80)	1 (20)	
	High	5 (71.4)	2 (28.6)	
X-ray	Results	N=32	N=6	0.027
	Normal	10 (66.7)	5 (33.3)	
	With radiographic findings	22 (95.7)	1 (4.3)	

DISCUSSION

Our retrospective study of admitted children with ARI tested with Respiratory Panel revealed a 76.6% prevalence rate of respiratory viruses. This finding is not surprising since most pediatric ARIs are of viral origin and the risk of concurrent (or subsequent) bacterial infection has been reported to be low in children over three months of age. As the agents of ARIs, viruses have constantly been shown to predominate.[8]

All of our subjects who tested positive for Respiratory Panel were due to viruses and no bacteria were detected. Our study showed similar findings of previous local study on acute LRTI in children that identified a higher proportion of viruses (27.2%) than bacterial agents (10.7%).[3] This is same as the study done among hospitalized children with ARI in China, wherein at least 1 virus was detected on 74.7% hospitalized children with ARI and only 22.2% had at least 1 bacteria detected.[9] Other studies regarding hospitalized children with ARIs had viral detection rates of 35-95%.[1,7] Possible explanations for the wide differences in detection rates in the literature include heterogeneity in study populations, differences in presenting respiratory symptoms, number of respiratory pathogens tested, method used for detection and genetic variability between populations.[1]

Children 5 years old and below comprise 72% of the total population for this study and most of the respiratory pathogens were detected in this age group. All RSV positive patients belong to this age group. RSV is responsible for more than 50% of cases of bronchiolitis and may also cause pneumonia especially in children less than 2 years old.[11] A local study done in 1989 supported the high incidence of ARI among Filipino children less than 5 years old in depressed urban communities in Metro Manila.[10] The higher detection rate of respiratory pathogens among infants and young children has been ascribed to a higher infection rate, lower viral clearance rate due to underdeveloped immune system and incomplete vaccinations. Furthermore, parents of younger children may seek healthcare earlier in the course of disease due to parental anxiety.[1]

Using the Respiratory Panel, RSV was the most frequent respiratory virus detected. In a local study, RSV was the second most frequently detected virus out of all ARI cases however, RSV was the most frequently detected among hospitalized patients.[12] The results of this study is comparable to several studies done abroad.[2,7,13,14] When categorized into upper and lower respiratory tract infections, the leading viral etiology of LRTI morbidity and mortality globally is RSV while RhV for URTI.[2,15] Symptoms of ARI include cough or difficulty of breathing, other signs and symptoms including fever, nasal obstruction/discharge, retractions, tachypnea, crackles, wheezes, rhinorrhea and sore throat. Our subjects presented mainly with LRTI reflected by the combination of cough and presence of retractions as the most common clinical manifestations. Acute cough in children is mostly caused by URTI but may also be a manifestation of serious conditions like bronchiectasis and the presence of chest wall indrawings identifies more severe disease like bronchiolitis and pneumonia which are LRTIs.[15,16] These findings may also be related to RSV being the most commonly detected respiratory pathogen in this study and that 60.5% of patients with chest x-ray had abnormal findings.

The presence of a sign or symptom of ARI did not show a significant relationship with the presence of a respiratory pathogen.[17] In cases of dual infections, a study found that fever and cough were the two main significant predictors for virus co-infection. Fever alone was a significant predictor for bacteria co-infection. Fever, cough, and sputum were significantly more frequent in virus and bacteria co-infection cases than monodetected.[9] Only 2 cases out of 36 patients with detected pathogens have viral co-detection in our study. Both Human Rhinovirus/Enterovirus and RSV were detected in one patient while Coronavirus 229E and hMPV in another. There were no viral-bacterial and bacterial co-infections. Among these 4 viruses, only Coronavirus 229E was not detected as a single pathogen for a patient with ARI. One study compared the clinical manifestations and laboratory tests for patients with negative detection, single infection, and co-infections. No statistically significant differences was found in terms of CRP level and CBC counts.[18]

Whether the detected pathogens are actually the cause of the respiratory symptoms or are simply colonizing the respiratory tract during symptomatic episodes remains unclear. It can be speculated that not every infection with a pathogen leads to respiratory symptoms and that pathogenicity might depend on host or environmental factors.[17] Primary infections with viral pathogens can predispose to secondary bacterial infections.[7] On the other hand,

vaccinations provide protection from vaccine preventable diseases (VPD) which in turn decrease the spread of related diseases, and improve child survival prospects (as children, particularly those under five years old, are more likely than adults to die from VPDs).[19] VPDs caused by *Bordetella pertussis, S. pneumoniae, H. influenzae type B, Corynebacterium diphtheriae*, measles virus and influenza virus frequently cause respiratory tract diseases.[20] The vaccination history of our study subjects was not looked into but this is an important aspect that should be explored as this may explain as to why no bacterial pathogen was detected in our subjects.

The possibility of detecting multiple targets in a single sample is particularly important when multiple different pathogens can cause the same clinical presentation. mPCR assays enable detection of an array of viruses with higher specificity, sensitivity, and faster turn-around time than previous testing using immunoassays or cultures.[21] Potential advantages also include conserving and optimizing analysis of other samples, simplify ordering algorithm as only one test needs to be requested, potential saving in reagents by testing multiple organism at once compared to testing each pathogen separately and standardized testing.[22] However, these assays also have their own limitations. Until now, implementation of multiplex molecular tests in clinical laboratories has been hindered by the high cost of the kits. Other disadvantages include false positive results due to cross reactivity or unspecific amplification caused by multiple primers/targets present in the reaction, false negative results due to use of preferential amplification of one target over the other, negative internal control due to exhaustion of reagents in samples with a high amount of one particular target.[22] Despite of these limitations, mPCR assays are being adopted rapidly in clinical practice.

The use of mPCR testing for respiratory viruses among hospitalized patients was significantly associated with decreased healthcare resource utilization including decreased use of chest radiographs.[21] Unlike CBC and CRP, a chest x-ray cannot differentiate between a viral or bacterial pathogen but an abnormal finding would mean a LRTI exists. This study showed that there is a higher probability of having a positive Respiratory Panel result when chest x-ray is positive however, 4.3% of patients will have a negative Respiratory Panel. A negative Respiratory Panel might be due to other bacterial pathogens not included in the panel. The prevalence of respiratory viruses in this study could enable approximation on the local epidemiology of respiratory infections and will influence physicians to decide on the management. Immunization status is also relevant because children fully immunized against *H. influenzae type b* and *S. pneumoniae* are less likely to have VPDs caused by these pathogens. However, in cases of diagnostic dilemmas like worsening of a disease severity despite of proper management and inconclusive laboratory and ancillary findings, Respiratory Panel is highly suggested. The utility of chest x-ray as a diagnostic tool for respiratory pathogens has not been established since there is limited evidence to support its routine use in distinguishing between viral and bacterial infections.[23] CRP, on the other hand, is one of the most frequently evaluated and indicative biomarkers for identifying bacterial infections in children because their levels are higher in bacterial infections than in viral infections.[24] In this study though, equivocal and high CRP were detected in half of the patients tested. This figure almost match the high CRP values in a study wherein 66.5% of patients with ARI was positive for a viral pathogen using a mPCR that detects 14 respiratory viruses and 59% of them had abnormal CRP.[8] Respiratory Panel can detect *Bordetella pertussis, Chlamydophila pneumoniae,* and *Mycoplasma pneumoniae* which are causes of atypical pneumonia. These organisms do not respond to penicillin derivatives, which may cause failure of antibiotic empirical therapy. Moreover, these fastidious bacteria are difficult to identify by culture or serology, and therefore often remain undetected. Thus, rapid and accurate identification of bacterial pathogens causing atypical pneumonia is crucial and need to be treated with macrolides.[25] However, the cost of a Respiratory Panel is still quite prohibitive compared to antibiotics and laboratory tests necessary for the

diagnosis and management of an atypical pneumonia and other ARIs.

The impact of mPCR on physician antimicrobial prescription practices remains unclear. In this study, we observed that antibiotics are prescribed too often. Among patients with ARI, 58.3% was already started on antibiotic and after the positive viral pathogen was relayed, 23.8% discontinued the antibiotic and 87.5% of patients with a negative Respiratory Panel result discontinued the antibiotic. In a study by Mcculloh, physicians started antibiotics more often in children with a negative Respiratory Panel result and occasionally discontinued antibiotics in children diagnosed with a viral pathogen.[26] These results suggest that Respiratory Panel testing may enhance physician decision-making when prescribing antimicrobials in children hospitalized with ARI. In another study, mPCR testing on admission was associated with less use of antibiotics compared with testing with non-mPCR based methods.[21] In a multicenter pediatric study, interviewing medical doctors on fictitious ARI cases, RT-PCR decreased antibiotic use. However, in real life, the same physicians did not alter their antibiotic prescriptions based on the results of RT-PCR.[27] The difference in findings could indicate that provider decisions for antibiotic use in the emergency department or ambulatory setting may be more impacted by clinical factors like physical examination or past medical history and less impacted by mPCR test results. In addition, in these settings, decisions are made within shorter time frames, and despite the relatively rapid turn-around time for mPCR testing, results may still not be timely enough to impact decision making.[21]

Most viral infections are supportively managed, hence rapid viral detection may help to make appropriate decisions and decrease unnecessary antibiotic use.[18] The precise diagnosis of certain viruses may contribute to timely antiviral agent treatment as well, like oseltamivir against influenza infection. In this study, Influenza A/H12009 is the most commonly detected pathogen in children 1-5 years old and the 2nd most commonly detected pathogen in all children admitted with ARI, but if all influenza virus variants will be combined, it turns out to be the most common virus in all ages. The Influenza A and B viral antigen rapid test has a sensitivity of 94.7% and specificity of 94% for influenza A, while a sensitivity of 91.7% and specificity of 97.5% for influenza B. This is almost the same as the Respiratory Panel having a sensitivity of 90-100% and specificity of 100% for influenza A, and 100% sensitivity and specificity for influenza B. Rapid diagnosis of influenza viruses and early treatment with oseltamivir is crucial.[28] Therefore, influenza rapid testing may be done instead for Respiratory Panel in detecting Influenza virus. It is a highly sensitive and specific test and relatively cheaper than the Respiratory Panel. The Centers for Disease Control and Prevention (CDC) also recommended that those patients who present with a syndrome consistent with influenza and have a negative rapid antigen test result should either receive a confirmatory RT-PCR test or be treated as if they have influenza.[29]

The guidelines of the Royal College of Paediatrics and Child Health (RCPCH) and the European Society of Paediatric Infectious Diseases (ESPID) recognize that RT-PCR is increasingly replacing immunofluorescence and serology, but they have not given recommendations when to use it and what the consequences are of the results when they become available.[27] One of the major drawbacks of the Respiratory Panel is its very high cost and despite the clinical impact of respiratory virus infections, its cost-effectiveness is incompletely understood.[29] Respiratory Panel mainly detects viruses and detects 3 bacterial species only, other common pathogens like *Streptococcus pneumoniae* and *Hemophilus influenzae* are not included in the panel hence single bacterial infections and co-infections that may modify the impact of respiratory pathogens on symptom could have not been detected. Hence, the researchers also looked on how the most common laboratory tests and the prevalence of respiratory pathogens may serve as a substitute for the Respiratory Panel.

CONCLUSION AND RECOMMENDATIONS

In conclusion, the prevalence of respiratory pathogens among admitted children with ARI in our institution during the study period showed 76.6% of children tested positive for Respiratory Panel and all of which were viruses. RSV was the most prevalent virus detected followed by Influenza A/H1-2009 and hMPV. Viral co-infections were detected in 4.3%. Due to similarities of viral and bacterial ARIs, empiric antibiotic may lead to antibiotic misuse. Respiratory Panel, as an emerging multiplex POCT system for the simultaneous detection of different pathogens, provides rapid and high-yield results which can guide diagnosis, therapy and infection control measures.[5]

This study was done in a comparatively small sample size and may be continued on a larger scale. The lack of statistical significance on some of the findings might improve with increase in number of subjects. Another limitation is that patients involved were admitted patients, implying that clinical manifestations are relatively more severe. Further studies may also include patients seen on an outpatient basis.

Some studies found high susceptibility for ARIs after natural viral infections. The detection of pathogens does not always mean that it is the cause of the current ARI but might be due to a previous or co-existing viral illness that could have predisposed to the current ARI. A better understanding of the etiological role of viral infections and the risk for subsequent ARIs is also needed for the prevention and management of childhood ARIs.

Lastly, future prospective studies to further assess the impact of Respiratory Panel on outcomes including arriving at a correct diagnosis, time to diagnosis, use or misuse of antibiotics, minimizing other diagnostic tests, length of hospital stay and clinical course is recommended.

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