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Molecular Epidemiology of Respiratory Viruses in Febrile Infants Under 90 Days Attending Pediatric Emergency Department

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Abstract Fever is one of the most common presenting complaints in paediatric emergency departments (ED). Acute viral respiratory infection is the most common findings. The aim of this study is to define types of respiratory viruses among febrile infants under 90 days attending to emergency department. Methods: In addition to sepsis workup, nasopharyngeal aspirates were collected from 265 febrile infants without an apparent source of infection. A multiplex PCR assay was used to detect 15 human viral species and subtypes. Results: Overall, 154/265 (58.1%) NPA specimens from febrile infants were positive for at least one human virus. Viral types detected were as follows: (60/265,22.6%) rhinovirus, (50/265,18.9%) respiratory syncytial virus, (28/265,10.6%) parainfluenza virus, (11/265,4.2%) influenza virus, (12/265,4.5%) coronavirus, (10/265,3.8%) metapneumo, (8/265,3%) adenovirus, (2/265,0.75%) enterovirus, and (2/265,0.75%) bocavirus. Co-detection of two viruses or more was also observed. Positive bacterial cultures were reported in 16.5%, 3.5%, and 2.8% of urine, blood and CSF samples respectively. Conclusion: Viral infections are frequent in febrile infants without an apparent source. Testing NPA for molecular identification of viruses in addition to the routine sepsis workup may help more accurate management of febrile infants. This could also limit the unnecessary use of antibiotics, and nosocomial spread of viruses, however, this needs to be further investigated.

Keywords: *febrile infants, under 90 days, respiratory viruses, molecular epidemiology, emergency department*

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1. Introduction

Fever is one of the most common presenting complaints in paediatric emergency departments (EDs), and it is estimated that around 17% of paediatric ED visits are attributable to a primary complaint of fever [1]. It was found that only around 10% of infants with fever may have serious bacterial infection (SBI) and the remaining infants were presumed to have a viral cause for fever [2,3,4,5]. Therefore, clinicians adapt an aggressive approach even for well looking infants who have no focus for infection for management of febrile infants under 90 days, especially in terms of hospitalization, and over-prescription of antibiotics [6,7,8,9]. Such approach has been attributed to the limitations in history and clinical examination of those infants, and limitations of diagnosis for identification of viral cause of disease [6,7,8]. Previously, the identification of viral cause of disease was through culture or fluorescent assays [10]. However, rapid high throughput diagnostic methods for identification of viral pathogens have been developed in recent years [11,12]. The multiplex polymerase chain reaction (PCR) technique has been reported to be highly sensitive and specific method for the simultaneous detection of a wide range of respiratory viruses [13,14].

Acute respiratory infections (ARI) are among the major causes of childhood mortality, and viral causes were found to be the most common [15]-[17]. There are limited data on the range of viral pathogen profiles of respiratory tract in infants younger than 90 days of age. Since a better understanding of the disease causes could assist optimizing health-care delivery to patients, therefore, we conducted this study to expand knowledge of the molecular epidemiology of respiratory viruses among febrile infants age 0-90 days attending to emergency department.

2. Materials and Methods

2.1. Patients

This prospective observational study was conducted in Paediatric ED (tertiary health-care), King Fahad Medical

City (KFMC) in Riyadh. Ethical approval for this study was obtained from Institutional Review Board of KFMC (IRB 10-073). The inclusion criteria for recruiting patients were febrile infants under 90 days of age present with rectal temperature of ≥38.0°C, or in the preceding days to paediatrics ED without an apparent source of infection. Exclusion criteria were patients having any of the chronic diseases (such as cystic fibrosis, chronic pulmonary disease, immunodeficiency, prematurity, and congenital heart disease), and those who received immunization (last 48 hrs) or antibiotics in the last five days. Informed written consent was obtained from one of the parents. Demographic data, and all other clinical data included were extracted from Patients parents during taking the medical history patients' medical record file. Two weeks after either leaving the ED or the hospital ward, follow up telephone calls were made to one of the parents in order to determine the clinical outcome of febrile infants included in the study.

2.2. Clinical samples

Nasopharyngeal aspirates (NPA) were collected from patients during ED consultation before receiving any further therapy, whether subsequently hospitalized or not. Multiplex PCR was performed within 24 h using Seeplex® RV15 multiplex detection kit which is a one-step reverse transcription PCR (RT-PCR) system. Demographic and other data regarding some associated respiratory symptoms were obtained from medical records. Cultures were done for CSF, blood, and urine in accordance with sepsis workup guidelines.

2.3. Molecular Detection of Respiratory Viruses

Viral nucleic acids were extracted using the MagnaPure total Nucleic Acid Kit according to manufacturer's instructions (Roche Applied Science). Multiplex PCR using Seeplex® RV15 ACE Detection kit (Seegene Inc., Seoul, South Korea) was performed for simultaneous detection of 15 human viral species and subtypes. This includes: Respiratory Syncytial A and B (RSVA, RSVB), Influenza A and B (FluA, FluB) Corona 229E/NL63 and OC43 (HCoV 229E/NL63, HCoV OC43), Parainfluenza 1-4 (PIV1-PIV4), Metapneumo (MPV), Rhino A/B/C (HRV), Adeno (AdV), Entero (HEV), and Boca 1/2/3/4 (HBoV).

2.4. Specimens for Culture

Blood, cerebrospinal fluid (CSF) and urine samples from patients were collected in suitable containers for the purpose of culture. These samples were processed in microbiology laboratory according to the guidelines of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) [18]. Pathogen identification and antimicrobial susceptibility testing was performed using an automated microbiology system (Phoenix; BD).

2.5. Statistical Analysis

Data were analyzed using the SPSS statistical software (version 16). Simple descriptive statistics and Pearson Chi-square tests were used for data analysis. The degree of statistical significance is denoted by the p value of 0.05.

3. Results

3.1. Patients and Prevalence of Respiratory Viruses

The total number of febrile infants included in this study was (265) with mean age of 41.8 (±23.4) days (from August 2011 to May 2013). Of them, 149/265 (56.2%) were male infants (Table 1). Gender stratified by age groups is demonstrated in Table-1 in addition to some other characteristics for the study population. In this study, we observed that 173/265 (65.3%) of febrile infants have received empiric antibiotics immediately after consultation. A highly significant difference between age groups is detected (p<0.000) for infants who empiric antibiotic prescriptions (Table 1). Only 51/265 (19.2%) of febrile infants included in this study had positive bacterial cultures, of them 18 were females and 33 were males. Overall, 252/265 (95.1%) of febrile infants in this study were completely recovered determined by follow up for the clinical outcome after 2 weeks (Table 1). If we exclude the missing data regarding the clinical outcome of 8 febrile infants seen in Table 1, the mortality rate in the study population is 1.2%.

Table 1. Description Of Certain Characteristics Of The Study Cohort

Variable	0-29 days 89 (100%)	30-59 days 85 (100%)	60-89 days 91 (100%)	Whole cohort 265(100%)
Age	89 (33.6)	85 (32.1)	91 (34.3)	265 (100)
Gender				
Male	47 (52.8)	53 (62.4)	49 (53.8)	149 (56.2)
Female	42 (47.2)	32 (37.6)	42 (46.2)	116 (43.8)
Infants with positive NPA	49 (55.1)	50 (58.8)	55 (60.4)	154 (58.1)
Infants with positive culture	21 (23.6)	15 (17.6)	15 (16.5)	51 (19.2)
Empiric antibiotics at first ED visit+				
Yes	76 (85.4)	54 (63.5)	43 (47.3)	173 (65.3)
No	13 (14.6)	31 (36.5)	48 (52.7)	92 (34.7)
Outcome*				
Recovery	81 (91.0)	83 (97.6)	88 (96.7)	252(95.1)
Complication	1 (1.1)	0 (0.0)	1 (1.1)	2 (0.8)
Death	0 (0.0)	2 (2.4)	1 (1.1)	3 (1.1)
Missing data	7 (7.9)	0 (0.0)	1 (1.1)	8 (3.0)

+ p<0.000 * Determined by follow up after 2 weeks.

3.2. Prevalence of Positive Bacterial Cultures From Routine Sepsis Workup

Table 2 demonstrates the type of clinical samples that were sent for culture as part of routine sepsis workup in addition to the NPA samples. The highest number of clinical samples that were sent for cultures were blood samples followed by urine and CSF (256, 260, and 142 respectively). Overall, 3.5% of blood specimens, 16.5% of urine specimens, and 2.8% of CSF specimens were positive for culture (Figure 1). When we stratify febrile infants having positive culture by age, we find that 41.2% of them fall in 0-29 days age, 29.4% of them fall in 30-59 days age, and 29.4% of them fall in 60-89 days age group (Figure 2). Tables 3 demonstrate the number of bacterial species that were identified in clinical specimens positive for bacterial cultures stratified by type of specimen. It is worth mentioning here that some of the febrile infants had bacterial growth in more than one clinical specimen, others have more than one type of bacterial growth in one clinical specimen. Overall, the number of bacterial stains isolated in clinical samples with positive culture is (60) (Table 3).

Table 2. Clinical Samples Sent For Cultures And NPA Performed For Febrile Infants

Test	0-29 days N (%)	30-59 days N (%)	60-89 days N (%)	Total
Blood culture	89 (100)	87 (97.6)	84 (92.3)	256
Urine culture	88 (98.9)	84 (98.8)	88 (96.7)	260
CSF culture	71 (79.8)	45 (52.9)	26 (53.6)	142
NPA	89 (100)	85 (100)	91 (100)	265

Table 3. Numbers Of Bacteria Detected In All Clinical Samples

Type of Sample++	Bacteria	No.
Urine*	E.coli	20
	E.coli (ESBL)	4
	Enterobacter aerogenes	3
	K. pneumonia	8
	Pseudomonas	3
	Enterococcus Faecalis	2
	S. agalactae	3
	S. aureus	2
	S. haemolyticum	1
	Candida	1
Total		47
Blood	E.coli	1
	K. pneumonia	1
	S. hominis	3
	E. faecalis	1
	Salmonella (non typhi)	1
	H. infuenzae (b)	1
	Streptococcus viridance	1
Total		9
CSF	Salmonella (non typhi)	1
	H. infuenzae (b)	1
	E.coli	1
	Enterococcus Faecalis	1
Total		4

*Two different bacteria were identified as cause of infection in three patients

++Some febrile infants had positive culture in more than one sample.

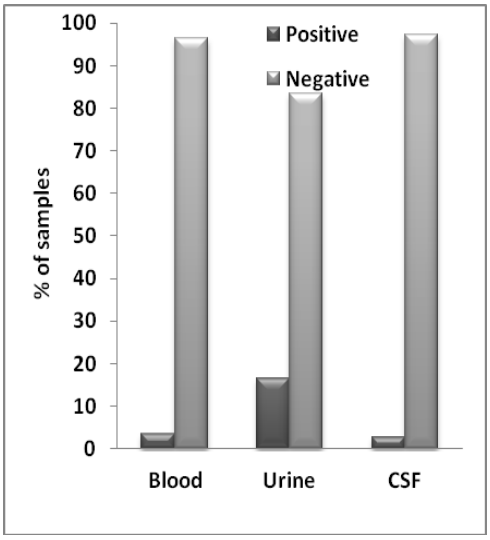

Figure 1. Percentage of clinical samples with positive culture results taken from febrile infants

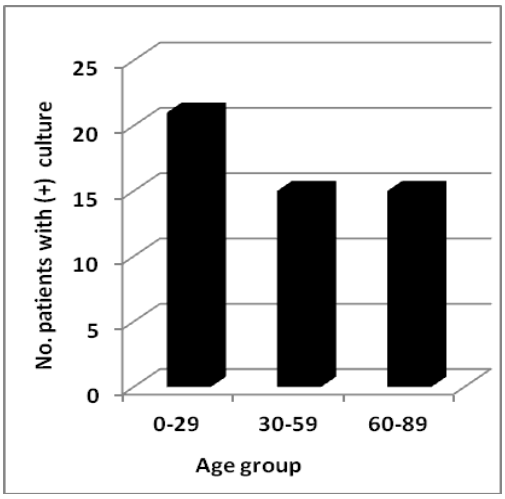

Figure 2. Number of febrile infants having positive cultures stratified by age

3.3. Detection of Viral Pathogens in Respiratory Tract

All febrile infants had their NPA samples tested by multiplex PCR (Table 2). Overall, we report 154 (58.1%) positive NPA specimens for at least one pathogen. Single virus detection is found in 127/154 (82.5%) patients with positive NPA. When we stratify the results of NPA by infants' age, we find positive presence of viruses in the respiratory tract of 49/89 (55.1%) of infants 0-29 days age, 50/85 (58.8%) of infants 30-59 days age, and 55/91 (60.4%) of infants 60-89 days age (data not shown). Figure 3 demonstrate types of viruses that were identified from respiratory tract of the study population. Overall, the numbers and percentages of viruses detected in the total 265 tested NPA samples are as follows: 60/265 (22.6%) HRV, 50 (18.9%) for all RSV, 28 (10.6%) for all PIV, 11 (4.2%) for all Flu viruses, 12 (4.5%) for all Corona viruses, 10 (3.8%) MPV, 8 (3%) AdV, 2 (0.75%) HEV, and 2 (0.75%) HBoV. The breakdown of subtypes in some groups of virus as a percentage was as follows: RSV (82% A, 18% B), PIV (50% PI3, 28.6% for PI1, 14.3% PI4, 7.1% PI2), Flu (90.9% A, 9.1% B), and Corona (91.7% OC43, 8.3% for 229E/NL63). The numbers and range of

viruses detected were also stratified by age groups of patients (Figure 3). The highest detection rate of viruses (35.7%) is observed in infants at the age group of 60-89 days. However, no significant difference can be detected between age groups with regard to the rate of virus detection ($\chi 2=61.7$, $p=0.627$).

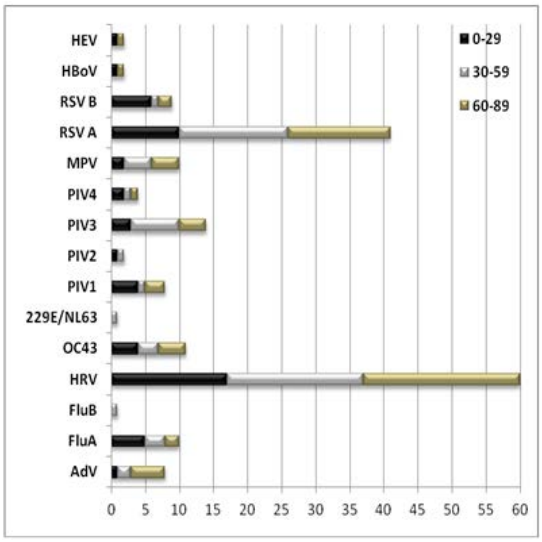

Figure 3. Types and numbers of viruses detected by multiplex PCR using NPA samples stratified by age group

3.4. Co-detection of Viral Pathogens

We report the co-detection of two viruses as 17 different combinations of virus groups in febrile infants with positive NPA 24/154 (15.6%) (Figure 4). The most prevalent virus in all dual co-detection is HRV and RSV [9/17 (52.9%), 6/17 (35.1%) respectively]. With exception of FluB, 229/NL63, and HBoV, all other viruses detected as single presence in this study, were also detected in dual presence. We also report the co-detection of triple viruses in 3/154 (1.9%) of febrile infants. Triple combinations are (AdV+PIV3+ HRV), (FluA+PIV4+ PIV3), and (RSVA+OC43+HRV). The first two combinations were in infants from age group of 30-59 days, the last was detected in infants from age group of 60-89 days.

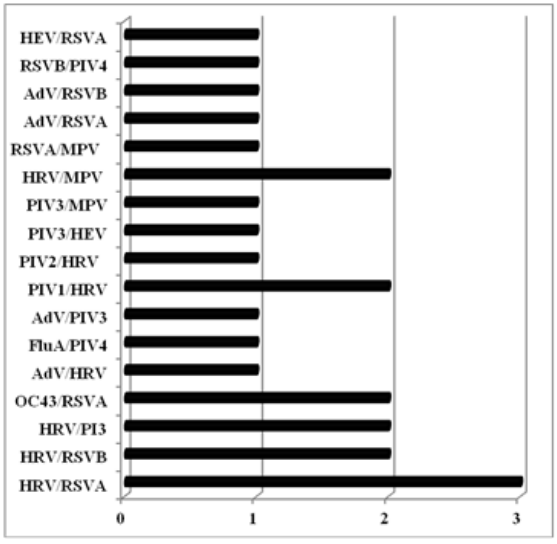

Figure 4. Types and numbers of dual viral combinations detected in febrile infants

4. Discussion

Infants 0-90 days age are susceptible to different set of pathogens, especially those under 2 months of age whom are incompletely vaccinated. Therefore, when infants present with fever, clinicians remain uncertain about the risk of SBI and start screening febrile infants even when there is no focus for infection [19]. Around 19% of febrile infants included this study had SBI, and the highest reported was urinary tract infection (UTI). This rate of SBI cases is higher than those were reported by other studies [20,21]. UTI occurring as the highest form of bacterial infection in infants and children in all ages has been similarly reported by other studies with E.coli being the most common causative pathogen [20,21,22,23].

Although only around 19% of all febrile infants in this study had positive cultures, around 65% of those infants have received empiric antibiotics for at least three days before results of culture appeared. Also we observed that, the younger is the age group the higher is the rate of antibiotic used. Interestingly, results of this study also revealed that nearly two thirds of patients had positive NPA for at least one virus. This rate of viral presence in respiratory tract is higher than that reported by Ganducci et al., [2008] which was around 47% [18]. However, authors in that study investigated children <2 years for the presence of only 4 types of viruses, while we targeted 15 virus species and subtypes. These differences will inevitably lead to increase the reported rate [18]. Multiplex PCR technology which has been reported to be a reliable method of detection [13,14].

The ability to detect HRV in NPA samples had increased substantially due to the molecular techniques [25]. HRV is predominant pathogen in premature infants and it may cause severe respiratory infections [26]. Our results indicated that HRV was the highest detected virus, and it formed around half of dual virus co-detections. Prevalence of HRV in this study is similar to those reported in USA where 54% of infant under 6 months were positive for HRV [25]. The association of HRV with respiratory infections has been reported for different age groups in other studies [27,28].

The incidence of RSV was the second most observed in this study with predominance of RSV A. This is not in agreement with other local and international studies which showed that the highest prevalence of respiratory viral infection was caused by RSV [24,29,30]. The difference in patients' age rang, in method of detection, and detection of other viruses such as HRV could be reasons behind this discrepancy. The co-detection rate of RSV in all dual viral presence (35.1%) is less than those reported in another study from China (76%) [31].

PIVs were the third most common viruses (28,10.6%), and the highest detected was PIV3 (50%). Generally, our findings are in agreement with local [29] and international reports from USA and Africa [32,33], however, in those studies patient age was less than five years old.

Flu virus was detected in 4.2% febrile infants, and FluA was the highest reported (90.9%) with one H1N1 infection (data not shown). During the epidemic in 2009, H1N1 caused 7.7% of respiratory infections in children < 2 years in France [28], 16% of children <16 years in Italy [30], and around 23% of children < 3 years old in Shanghai [34]. Locally, 11% and 7% of cases of bronchitis in children <

5 years were reported to be caused by Flu virus A and B (respectively) [29].

HCoV is known to cause acute respiratory infections in children younger than 5 years [35]. The Seeplex kit was not designed to detect the novel Corona virus which was circulating as sporadic cases around the time we were at the end of this study. We reported (4.5%) of febrile infants had HCoV and the majority were of OC43. HCoV was reported in 8.7% of children under 2 years with acute respiratory disease in Italy (around 40%, 36% were OC43 and NL63 respectively) [34]. HCoV infection reported to be around 5% in children under 3 year with respiratory infection in China [34], while in USA, most reported HCoV positive children were under 2 years age and 47% of them were infants <6 moths. The highest prevalent strain in latter study was NL63 and the least was CO43, 229E was not detected [35]. Although our rate of detection of HCoV is nearly similar to other reports, however, there is a difference in the distribution of different strains.

MPV was discovered in the last two decades and are reported to cause respiratory disease similar to those caused by RSV [36]. MPV was detected in 3.8% of our study population. This incidence is lower than those reported in Italy (14.3%) in children < 2 years [24], and higher than those reported in China (0.6%) in children <3 years and around 6% in children <13 years (18,38). The incidences observed for AdV (3%), HEV (0.75%), and HBoV (0.75%) were less than those reported in older children elsewhere in the globe [24,34,37].

Finally, we believe that a better understanding of the disease causes could help optimize health-care delivery to patients. The rapid detection of one respiratory virus has been found to affect physician decision-making regarding the decrease in prescription of antibiotic [38]. We hope that our results will have a similar or even a better effect in enhancing the management of febrile infants under 90 days. We also need to report limitations of this study, and one of the important limitations was the difficulty in recruiting patients due to the nature of the NPA sample. This study was conducted in a tertiary hospital care in one medical centre which may also be reflected on the limited numbers of patients.

5. Conclusion

Overall, this study highlights the molecular epidemiology of a wide range of respiratory viruses that are detected in febrile infants under 90 days age attending paediatric ED. Viral infections are frequent in febrile infants without an apparent source. We suggest that, testing NPA for molecular identification of viruses in addition to the routine sepsis workup may help more accurate management of febrile infants by ED physicians. This could also limit the unnecessary use of antibiotics, hospitalization, and nosocomial spread of viruses, however, this needs to be further investigated.

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Declaration of Interest

The authors have no competing interests.

List of Abbreviations

1. Influenza A and B viruses (FluA, FluB)
2. Respiratory syncytial virus A and B (RSVA, RSVB)
3. Adenovirus (AdV)
4. Metapneumovirus (MPV)
5. Coronavirus (HCoV 229E/NL63), and corona virus (HCoV OC43)
6. Parainfluenzaviruses 1-4 (PIV1-PIV4)
7. Rhinovirus (HRV)
8. Enterovirus (HEV)
9. Bocavirus (HBoV).

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