ECRONICON OPEN ACCESS

EC PAEDIATRICS
Research Article

Etiology of Respiratory Infections among Neonatal and Pediatric Inpatients in a Tertiary Hospital in Makkah, Saudi Arabia

Sami Melebari[1*], Abdul Hafiz[2], Saied Dehlawi[1], Fayez S. Bahwerth[3], Asim Khogeer[4], Zainularifeen Abduljaleel[5], Fadel Qabbani[1], Ashwaq Hakim[1], Mohamed Bazaid[1] and Mohammed Kurdi[1]

[1]Department of Molecular Biology, The Regional Laboratory, Ministry of Health (MOH), Makkah, Saudi Arabia

[2]Department of Medical Parasitology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

[3]Department of Microbiology, Hera General Hospital, Ministry of Health (MOH), Makkah, Saudi Arabia.

[4]Department of Research and Plan, General Directorate of Health Affairs Makkah Region, Ministry of Health (MOH), Makkah, Saudi Arabia

[5]Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

***Corresponding Author:** Sami Melebari, Department of Molecular Biology, The Regional Laboratory, Ministry of Health (MOH), Makkah, Saudi Arabia.

Received: December 10, 2021; **Published:** February 23, 2022

Abstract

Acute respiratory infections (ARIs) are a common occurring infection in neonatal and pediatric inpatients. To determine the incidences and etiology of ARIs, we carried out a cross sectional study in a tertiary hospital in Makkah. We recruited a total of 166 children with symptoms of ARIs. Using bacterial culture techniques and polymerase chain reaction (PCR) based FilmArray RP2 plus, we detected bacterial and viral pathogens in the samples collected from these patients. Only 80 of our samples were infected with a viral or bacterial pathogen as per our diagnosis. Respiratory Syncytial Virus (RSV) was the most common pathogen present in our samples. We found that RSV infection in our patients mostly manifested as unspecified sepsis, neonate jaundice, respiratory distress and acute bronchitis.

Keywords: *Respiratory Infections; Bio Fire Film Array RP2 Plus; Pediatrics; Infectious Disease*

Introduction

Globally, the acute respiratory infections (ARIs) are known as one of the most important causes of morbidity and mortality. The prevalence was estimated at around 2.2 million of deaths from ARI happen during the world every year[1]. The majority of these infections include the upper respiratory tract (URT); however, infections of the lower respiratory tract (LRT) are also common. Fungi and bacteria can cause the acute respiratory infections, but viral infections are the most common, particularly in the pediatric age group [2]. Well known respiratory viral pathogens include Respiratory Syncytial Virus (RSV), Enteroviruses (EVs), Rhinoviruses (RVs), Influenza Virus (INF-A/B), Human Metapneumovirus (hMPV), Parainfluenza Virus (PIV-1/2/3), and Adenoviruses [3]. Recently, several viruses have been detected in patients with respiratory infections, such as human coronaviruses (hCoVs) and human polymaviruses [4].

Correct diagnosis of acute respiratory infections has been proven to help reduce misuse of the antibiotics as well as the length of stay in the hospital. It is well known that the traditional methods, such as the *invitro* culture and direct immunofluorescence assays may take longer duration to provide diagnostic results to the physicians, and are technically demanding as well [5]. The clinical examination and the symptoms associated with acute respiratory infection may be similar in several pathologies of the viral infection, which may cause difficulty in real diagnosis of the disease, as well as in determining the appropriate treatment. Nevertheless, challenges in detecting respiratory viruses include inconsistent clinical manifestations of viral RTI's and variable sensitivity and specificity of available diagnostic assays [6,7].

The ability to detect viruses has been improved in past few years. Several types of molecular biological procedures, including real time PCR, PCR-hybridization, reverse transcription PCR (RT-PCR) and multiplex PCR have been found as more fast and sensitive detection methods for respiratory infections [8,9]. Recently, our laboratory has used FilmArray Respiratory Panel (RP2+); an automated multiplex polymerase chain reaction system for rapid detection of 22 respiratory pathogens; 18 viral pathogens and 4 bacterial species [10]. It can be detected in a closed system directly from the nasopharynx (NP) or oropharynx (OP) samples. The method is one of the fastest way to results that needs only 2 minutes of hands-on time, and approximately 45 minutes of instrumentation time [11]. Hence, the goals of our study is to examine the incidences of various pathogens that causes ARIs in Makkah city with Acute respiratory tract infections (ARTIs) and to assess the presenting clinical, epidemiological, and demographic characteristics of these different infections. The study depends on the results got from the BioFire® FilmArray® Respiratory Panel 2 plus (RP2+) assay as well as bacterial culture method for detection of pathogens.

Materials and Methods

Ethical approval

Ethical approval was achieved from ethics committee of Ministry of Health in Kingdom of Saudi Arabia (Ethical approval reference No: H-02-K-076-1120-415). All data was collected with before consent to conduct the study from the Ministry of health, The Kingdom of Saudi Arabia. Patient's data collected from the regional laboratory and Hera hospital in Makkah was anonymized and analysed for this study.

Study area and sample collection

The study was conducted during the influenza season between November 2018 and March 2019, in Makkah, Kingdom of Saudi Arabia. Makkah is the holiest city in Islam and the capital of the Makkah Province in Kingdom. In 2020, the estimated recorded population is 2,042,000, which bring Makkah as the 3rd city with the most populate in Saudi Arabia [12]. Patients presented with symptoms such as lower and/or upper respiratory tract infection with fever (≥ 38°C), cough, bronchitis, pneumonia and/or a sore throat were recruited in the study. The demographic, clinical data and laboratory tests results were taken from records including symptoms, gender, age, medical condition, physical examinations, laboratory tests and clinical diagnosis.

Bacteriological examination

Each swab plated on Blood the agar and MacConkey agar (Difco Laboratories, Detroit, MI, USA). The plates then incubated at 37°C for around 24-38 hrs. The suspected colonies were examined using biochemical and serology tests. After plated, the samples were examined for viruses using the FilmArray® Respiratory Panel 2 *plus* (RP2+).

Film array RP2+ Analyses

Approximately 300ul from the sample was transfer to the FilmArray RP2+ panel testing according to the manufacturer's instructions [13]. All samples were handled in a biosafety cabinet and done with operators wearing the suitable personal protective equipment. The test of FilmArray RP2+ contains of automated nucleic acid extraction, nested multiplex PCR, nucleic acid amplification [14]. The test also takes around 45 min per run in each specimen and the result is reported as "detected" or "not detected" with each target. Also, If the internal control is failed, the software automatically gives to all panel analysis a result of "invalid". The panel of this FilmArray is high sensitivity (85% - 100%) and specificity (95% - 100%) [15].

The FilmArray RP2+ panel identifies for twenty-two pathogens (4 bacteria and 18 viruses) that responsible for respiratory tract infections. These pathogens including the coronavirus (strains MERS-CoV, NL63, HKU1, 229E, OC43); adenovirus, rhinovirus/enterovirus; human metapneumovirus; RSV; influenza (strains A, A/H1, A/H3, A/H1-2009, B); and parainfluenza virus (strains 1, 2, 3, 4); as well as the bacterial respiratory pathogens Bordetella (IS 1001, ptxP), Mycoplasma and Chlamydophilia [16,17].

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25.0 (SPSS Inc., Chicago, IL, USA), and we were using Chi-square test for qualitative data.

Results

Patients characteristics

A total of 80 children with at least one detected viral or bacterial pathogen and 86 children without any detected viral or bacterial pathogen were recruited in this study. The pathogen (+ve) group are those whose laboratory report confirms at least one bacterial or viral infection. The Pathogen (-ve) group were those recruits that have laboratory report negative for viral and bacterial infections. Younger age was a risk factor for infections in our study subjects with very small *p* value. Gender and nationality did not have any significant association with pathogen (+ve) or pathogen (-ve) group in our study. We observed the *p* values 0.83 and 0.85 for gender and nationality, respectively.

Incidences of viral and bacterial infections

In the pathogen (+ve) group, the most prevalent infectious agent detected was Respiratory Syncytial Virus (n = 64) accounting for about 80% of our cases. RSV was followed by Human Rhinovirus/Enterovirus (n = 13) accounting for about 16.3% of our patients. Parainfluenza virus 4 was the third most prevalent virus that was detected in 5% of our patients (n = 4). The rest virus infections had a prevalence of less than 5%. In addition, about 16.3% of our patients (n = 13) had no viral infections detected by Film Array machine. Among pathogenic bacteria, *Klebsiella pneumoniae* was the most prevalent bacterial infection detected by culture method (n = 9, 11.3%), followed by Methicillin-resistant *Staphylococcus aureus* (MERS) (n = 4, 5%). Rest of the bacterial infections, such as Acinetobacter baumannii, Staphylococcus aureus and Pseudomonas aeruginosa had prevalence of less than 2.5%.

Distribution of patients in different wards

Analysis of data with different wards suggests that there is no difference between pathogen (+ve) group and pathogen (-ve) group, in terms of ward. Neonatal Intensive Care Unit (NICU) contributed 70 subjects in pathogen (+ve) group and 73 subjects in pathogen (-ve) group. Pediatric ward contributed 3 subjects in pathogen (+ve) and 5 subjects in pathogen (-ve) group. Female surgical ward (FSW) and labor ward each contributed 2 cases in each patients group. Male surgical ward contributed 2 individuals in pathogen (+ve) and 3 individuals in pathogen (-ve) group. Whereas obstetrics and gynecology (OBG) ward contributed subject one in each group. On statistical analysis, we did not find any evidence that a particular ward is more likely to contribute to pathogen (+ve) or pathogen (-ve) group in our study.

Distribution of viral and bacterial infections in different types of patients' samples

To test if a particular type of sample is more likely to contain pathogens, we used statistical analysis to compare the sample distribution among pathogen (+ve) and pathogen (-ve) groups. A total of four types of samples were collected from our study subjects, i.e. nasal, throat, cerebrospinal fluid and pleural fluid. Half of the pathogen (+ve) samples were nasal samples (n = 40) while 47.7% of the pathogen (-ve) samples were nasal samples (n = 41). The throat samples made up of 46.3% of pathogen (+ve) samples (n = 37) and 47.7% of the pathogen (-ve) samples (n = 41). The cerebrospinal fluid contributed 3 subjects in each group amounting to 3.8% of the pathogen (+ve) and 3.5% of the pathogen (-ve) groups there was only a single sample of pleural fluid origin in the pathogen (-ve) group accounting for 01.2% of all samples in the pathogen (-ve) group. There was no evidence of differential distribution of the samples between the two groups.

The prognosis of RSV patients

Since RSV was the most prevalent virus in our patients, we wanted to analyse its prognosis by physicians. Unspecified sepsis was the most common prognosis in RSV patients, being present in 29 patients (73.3%) and absent in 9 patients (23.7%). Seven RSV patients were classified as having neonate jaundice while none of the RSV negative patients had this prognosis. Similarly, respiratory distress was identified in 6 RSV positive patients while this condition was absent in all RSV negative patients. Acute bronchitis was prognoses in 5 RSV positive patients while it was absent in all RSV negative patients. This differential prognosis fell slightly short of statistical significance (p = 0.057).

		Groups				Total		Chi-square	
	Pathogen +ve		Pathogen -ve						
	N	%	N	%	N	%	X^2	*p*-value	
Age	**1-6M**	28	35.00%	14	16.30%	42	25.30%	85.774	0.00
	6-1Y	49	61.30%	14	16.30%	63	38.00%		
	1Y-5Y	3	3.80%	58	67.40%	61	36.70%		
Gender	**F**	32	40.00%	33	38.40%	65	39.20%	0.046	0.83
	M	48	60.00%	53	61.60%	101	60.80%		
Nationality	**Saudi**	75	93.80%	80	93.00%	155	93.40%	0.035	0.85
	Non-Saudi	5	6.30%	6	7.00%	11	6.60%		

Table 1: *Patients characteristics.*

Virus		
	N	%
Respiratory Syncytial Virus	64	80
Human Rhinovirus/Enterovirus	13	16.3
Parainfluenza Virus 3	3	3.8
Parainfluenza Virus 4	6	7.5
Coronavirus NL63	2	2.5
Human Metapneumovirus	2	2.5
Human parachovirus	1	1.3
Streptococcus pneumoniae	2	2.5
Bordetella pertussis(ptxP)	1	1.3
Influenza B	1	1.3
No virus	13	16.3
Culture result		
Normal flora	56	70
No Bacterial growth	6	7.5
Klebsiella pneumoniae	9	11.3
Methicillin-resistant *Staphylococcus aureus* (MERS)	4	5
*Acinetobacter baumanni*i	2	2.5
Staphylococcus aureus	2	2.5
Pseudomonas aeruginosa	1	1.3

Table 2: *Incidences of virus and bacterial infections.*

Ward	Groups				Total		Chi-square	
	Pathogen (+ve)		Pathogen (-ve)					
	N	%	N	%	N	%	X^2	P-value
NICU	70	87.50%	73	84.90%	143	86.10%	0.553	0.99
PED	3	3.80%	5	5.80%	8	4.80%		
FSW	2	2.50%	2	2.30%	4	2.40%		
Labour ward	2	2.50%	2	2.30%	4	2.40%		
MSW	2	2.50%	3	3.50%	5	3.00%		
OBG	1	1.30%	1	1.20%	2	1.20%		

Table 3: *Ward wise distribution of study subjects.*

Sample type	Groups				Total		Chi-square	
	Pathogen (+ve)		Pathogen (-ve)					
	N	%	N	%	N	%	X^2	P-value
Nasal	40	50.00%	41	47.70%	81	48.80%	1.387	0.709
Throat	37	46.30%	41	47.70%	78	47.00%		
CSF	3	3.80%	3	3.50%	6	3.60%		
Pleural fluid	0	0.00%	1	1.20%	1	0.60%		

Table 4: *Types of sample distribution in study subjects.*

Physicians diagnosis/prognosis	Respiratory Syncytial Virus			
	-ve		+ve	
	N	%	N	%
ACUTE BRONCHITIS	0	0.00%	5	100.00%
BRONCHITIS	2	33.30%	4	66.70%
PREGNANCY RELATED-CONDITION	1	33.30%	2	66.70%
FEBRILE CONVALSION	2	100.00%	0	0.00%
FEVER UNSPECIFIED	2	66.70%	1	33.30%
NEONATE JUNDICE	0	0.00%	7	100.00%
BRONCHOPNEUMONIAE	0	0.00%	2	100.00%
PNEUMONIAE	0	0.00%	2	100.00%
UPPER RESPIRATORY TRACT INFECTION	0	0.00%	2	100.00%
SEPSIS UNSPECIFIED	9	23.70%	29	76.30%
RESPIRATORY DISTRESS	0	0.00%	6	100.00%
PAIN UNSPCEFIED	0	0.00%	1	100.00%
BIRTH ASPHYXIA	0	0.00%	1	100.00%
RESPIRATORY ABNOEA	0	0.00%	1	100.00%
VOMITING	0	0.00%	1	100.00%
Total	16	20.00%	64	80.00%
p value	0.057			

Table 5: *The prognosis of RSV.*

Discussion

Acute respiratory infections (ARIs) are one of the most important causes of morbidity and mortality among children below 5 years of age. ARIs are caused by bacterial as well as viral infections. The majority of ARIs include the upper respiratory tract (URT) infections;

however, infections of the lower respiratory tract (LRT) are also common. Correct identification of pathogen causing ARIs is important for treatment and management of the disease as well as to stop misuse of antibiotics.

This study examined the etiology of ARIs in a tertiary hospital in Makkah. Interestingly, we did not detect any pathogenic organism in about half (n = 86) of our patients despite these patients were having symptoms of infections. There can be several possible explanations of this observation. Firstly, our detection techniques did not detect the pathogens in these 86 patients. Including more pathogen detection panels in the Biofilm array machine may be helpful in such cases. Also, the culture techniques used in this study might have missed certain bacterial and fungal pathogens. For instance, the bacteria *Microbacterium tuberculosis* requires special culture techniques, which was not used in our study.

However, in this study the high range of infection was caused by several species of viruses, and this finding is like as previous research papers, that notified viral detection rates of 47 - 95% in children [19,20]. In addition, the higher detection rate of respiratory pathogens was found in this study among infants and young children, and this could be probably due to the immune and respiratory systems are developing in these young children, which maybe more susceptible to respiratory pathogens.

The Respiratory Syncytial Virus (RSV) is the most common pathogen detected in our study. It is a single stranded, negative-sense, non-segmented RNA virus. RSV belongs to the Pneumovirinae subfamily of the Paramyxoviridae family. World Health Organization (WHO), estimations suggest that RSV infects almost all children by the second year of their live resulting in 64 million clinical cases and 160,000 deaths annually. In our study 64 of the 80 pathogen positive samples had RSV virus in them accounting for 80% of the pathogen positive group. RSV is known to exist in two subtypes A and B[18]. We could not determine how differently these subtypes were distributed in our study subjects. However, in further studies, it will be interesting to see the distribution of these RSV subtypes and if they are associated with differential prognosis or differential patient outcome.

In order to understand the possible symptomatic manifestation of RSV infection, we analyzed the physician's diagnosis of RSV patients. We found that RSV infections in our patients were mostly manifested as unspecified sepsis, neonate jaundice, respiratory distress and acute bronchitis. Larger studies are needed to evaluate if these symptoms have prognostic value for RSV infection.

To test if certain infections are more common in different wards of Hira hospital, we analyzed if patients coming from NICU, PED, FSW, Labor ward, MSW and OBG wards have differential distribution between pathogen +ve and pathogen –ve groups. However, we did not find any significant difference. Similarly, we tested if the sample type has any effect on being pathogen +ve or pathogen –ve in our study. We compared nasal, throat, CSF and Pleural fluid for being differentially distributed between pathogen +ve and pathogen –ve groups. We did not find any significant difference in this regard.

Our study has limitations, as it is a single hospital study. Multiple similar studies are needed to determine the incidences of ARI in children in Saudi Arabia. Moreover, more than half of our study patents were negative for any pathogen, despite having fever and symptoms of respiratory infections. More robust diagnostic techniques are required to determine the pathogens underlying these infections.

Conclusion

This study reports the etiology of respiratory infections among neonatal and pediatric inpatients in a tertiary hospital in Makkah. The high incidence rates of viral infections and its clinical effect highlight the essential to perform a systematic surveillance program in Makkah for a superior management of acute respiratory infections in children. In addition, the importance of RSV should be taken as concern by doing more investigation as it was considered the most organism in this study found caused infection to the young children.

Acknowledgements

The authors are grateful to the Hera laboratory that has contributed to the success of this study.

Bibliography

1. Bakir TMF., *et al*. "Viral Aetiology and Epidemiology of Acute Respiratory Infections in Hospitalized Saudi Children". Departments of Pathology and Medicine, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia (1998).
2. Al-Hadramy M., *et al*. "Acute lower respiratory tract infections in Jeddah". *Saudi Medical Journal* 9 (1988): 34-39.
3. Bicer S., *et al*. "Virological and clinical characterizations of respiratory infections in hospitalized children". *The Italian Journal of Pediatrics* 39 (2013): 22.
4. Albuquerque M., *et al*. "Novel respiratory virus infections in children". *The Brazilian Journal of Infectious Diseases* 15 (2009): 806-808.
5. Advani S., *et al*. "Detecting respiratory viruses in asymptomatic children". *The Pediatric Infectious Disease Journal* 31 (2012): 1221-1226.
6. Huijskens E., *et al*. "Diagnostic value of respiratory virus detection in symptomatic children using real-time PCR". *Virology Journal* 9 (2012): 276.
7. Bicer S., *et al*. "Virological and clinical characterizations of respiratory infections in hospitalized children". *The Italian Journal of Pediatrics* 39 (2013): 22.
8. Bibby D., *et al*. "Comparative evaluation of the Seegene Seeplex RV15 and real-time PCR for respiratory virus detection". *Journal of Medical Virology* 83 (2011): 1469-1475.
9. Culebras E., *et al*. "Detection and genotyping of human respiratory viruses in clinical specimens from children with acute respiratory tract infections". *Revista Española de Quimioterapia* 26 (2013): 47-50.
10. Guillot S., *et al*. "Low Detection Rate of Bordetella pertussis Using the BioFire FilmArray Respiratory Panel 2plus. Biodiversity and Epidemiology of Bacterial Pathogens, Institute Pasteur, Paris, France (2020).
11. Chiua S., *et al*. "Surveillance of upper respiratory infections using a new multiplex PCR assay compared to conventional methods during the influenza season in Taiwan". *The International Journal of Infectious Diseases* 61 (2017): 97-102.
12. Mecca, Saudi Arabia Metro Area Population 1950-2020.
13. The BioFire® FilmArray® System.
14. Leber A., *et al*. "Multicenter Evaluation of BioFire FilmArray Respiratory Panel 2 for Detection of Viruses and Bacteria in Nasopharyngeal Swab Samples". *Journal of Clinical Microbiology* 56.6 (2018): e01945-17.
15. Protocols for Laboratory Verification of Performance of the FilmArray® Respiratory Panel 2 (RP2).
16. Subramony A., *et al*. "Impact of Multiplex Polymerase Chain Reaction Testing for Respiratory Pathogens on Healthcare Resource Utilization for Pediatric Inpatients". *The Journal of Pediatrics* 173 (2016): 196-201.

17. BioMérieux.

18. Mufson M., *et al*. "Two distinctsubtypes of human respiratory syncytial virus". *Journal of General Virology* 66 (1985): 2111-2124.

19. Al-Ayed M., *et al*. "Viral etiology of respiratory infections in children in southwestern Saudi Arabia using multiplex reverse-transcriptase polymerase chain reaction". *Saudi Medical Journal* 35 (2014): 1348-1353.

20. Assane D., *et al*. "Viral and Bacterial Etiologies of Acute Respiratory Infections Among Children Under 5 Years in Senegal". *Microbiology Insights* 11 (2018): 1178636118758651.

Volume 11 Issue 3 March 2022