



PREVALENCE OF SOME PATHOGENS DETECTED BY MULTIPLEX REAL-TIME PCR IN HOSPITALISED CHILDREN WITH ACUTE RESPIRATORY INFECTIONS IN BAC GIANG PROVINCIAL GENERAL HOSPITAL

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Objectives: Investigate the infection rate of some microorganisms using multiplex real-time PCR techniques in inpatient children with acute respiratory infection (ARI) in Bac Giang Provincial General Hospital.

Subjects and methods: A retrospective cross-sectional descriptive study. There were 450 cases ARI children treated at the Pediatrics Department in Bac Giang Provincial General Hospital though medical records with multiplex real-time PCR results of nasopharyngeal swab testing using both RP1 and RP4 kits were included in the study.

Results: Among 450 ARI children, the age group of under 60 months old accounted for the largest rate (81.6%). Influenza virus and RSV caused infection for infant and all ages group, focus on 2-60 months old group. The rate of pathogens detection using RP1 kit was 23.8% and the influenza infection rate was 13.6%, RSV was 10.2%. The rate of bacteria detected by RP4 kit was 40.0%. *S. pneumoniae*, *H. influenzae* infection were found across all age group, focus on children under 5 years old. The rate of *S. pneumoniae* infection was 24.4% and *H. influenzae* infection was 25.3%. *M. pneumoniae* infection was 2.4%, and such atypical pathogens mainly caused disease in the over 2 years old group. Some pathogens have low infection rate: *B. pertussis* (0.2%), *L. pneumophila* (0.2%), *C. pneumoniae* (0.2%). Combining RP1 and RP4 kits could enhance the detected rate of the ARI pathogens to 53.8%. 10.0% of co-infections were detected. Influenza infection rate was highest in spring (10.5%), decreased in summer and autumn, and gradually increased in winter (5.6%). RSV infection rate was highest winter (5.6%). *S. pneumoniae* and *H. influenzae* infections were distributed equally over the year but the peaks were found in November 2020 (7.1% - 6.0% respectively) and January 2021 (5.8% - 6.9% respectively). The highest rate of *M. pneumoniae* infection was in April 2021 (1.8%).

Conclusions: Kit RP1 could detect 23.8% respiratory pathogens, of which 13.6% were influenza; 10.2% RSV. There were 40.0% positive for at least one pathogen in the RP4 kit, including 24.4% *S. pneumoniae*, 25.3% *H. influenzae*, 2.4% *M. pneumoniae*, 0.2% *B. pertussis*, 0.2% *L. pneumophila*, 0.2% *C. pneumoniae*. Combining RP1 and RP4 kit could enhance the positive rate to 53.8% including 13.8% were infected with 1 kind of virus, 30.0% were infected with 1 kind of bacteria and 10.0% were co-infection. The co-infection patterns still remain unclear and could be a result of random combination. Influenza, RSV and *M. pneumoniae* infections were significant affected by seasoning, while *S. pneumoniae* and *H. influenzae* infections were sporadic all over the time.

Keywords: RP1, RP4, ARI, Multiplex real-time PCR.

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INTRODUCTION

Acute respiratory infection (ARI) is a common disease in childhood caused by many different viral and bacterial etiologies, and causes the death of 15 million children under 5 years of age/year worldwide^{[1],[2]}. Accurate, prompt and complete determination of the pathogens of ARI is essential for

treatment and prevention. To detect viral pathogens and bacterium, we can use rapid tests, immunoassay, culture and PCR, but the each method has some disadvantages, each technique only allows to determine 1 pathogens. However, performing many techniques on one patient will lead to increased expenditure and be time-consuming. Currently, multiplex real-time PCR has been developed and used to accurately identify specific gene segments of many microorganisms in one test, allowing detection and identification of many microorganisms simultaneously.

In Bac Giang Provincial General Hospital, a multiplex real-time PCR has been used to identify several viruses and bacteriae from the respiratory tract using kits from Seegene Company - Korea (IVD certificate). The producer developed four kits to detect and identify many respiratory pathogens. RP1 kit determinates influenza (type A, subtype A-H1, A-H1N1 epidemic 2009 type, A-H3, influenza type B), and respiratory syncytial virus - RSV (type A and type B); RP2 kit detects *Adenovirus*, *Metapneumovirus*, *Enterovirus*, *Parainfluenzavirus 1*, and *Parainfluenzavirus 2*; RP3 kit detects *Bocavirus* (type 1, 2, 3, 4), *Rhinovirus* (type A, B, C), and *Coronavirus* (type 229E, NL 65, OC 43); and RP4 kit detects *M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*, *H. influenzae*, *S. pneumoniae*, *B. pertusis*, and *B. parapertusis*. However, in the fact, the clinicians usually use 2 kits of RP1 and RP4. To evaluate the effectiveness of these two kits, we investigate the prevalence of some pathogens that cause ARI in children using multiplex real-time PCR RP1 and RP4 kits.

SUBJECTS AND METHODS

Subjects: ARI children treated at the Pediatrics Department in Bac Giang Provincial General Hospital though medical records with multiplex real-time PCR results of nasopharyngeal swab testing using both RP1 and RP4 kits from October 1, 2020 to September 30, 2021.

*Criteria for selection: Medical records of inpatient children diagnosed with ARI at the Pediatrics Department in Bac Giang Provincial General Hospital, with sufficient results of multiplex real-time PCR using both RP1 and RP4 kits to detect some microorganisms.

*Exclusion criteria: ARI children has result of nasopharyngeal swab testing using both RP1 and RP4 kits but do not have enough information as required by this study medical record form.

Methods:

Study design: A retrospective cross-sectional descriptive study.

Sample size: 450 ARI children with criteria-satisfied medical records from October 1, 2020 to September 30, 2021.

Information collection methods: Extract medical record informations, fill out informations collection form and import data into SPSS 16.0 software.

Data analysis using SPSS 16.0 software.

RESULTS

There were 450 cases ARI children treated at the Pediatrics Department in Bac Giang Provincial General Hospital though medical records with multiplex real-time PCR results of nasopharyngeal swab testing using both RP1 and RP4 kits from October 1, 2020 to September 30, 2021 with some of the following results:



Relation between age of ARI children and percentage of pathogen-induced infection

Table 1. Age of ARI children and percentage of pathogen-induced infection

Agents	ARI number		Influenzae		RSV		SP		HI		MP	
	n	%	n	%	n	%	n	%	n	%	n	%
Ages												
Infant	24	5.3	2	8.3	5	20.8	7	29.1	7	29.1	0	0.0
2 - 12 months	120	26.7	9	7.5	16	13.3	27	22.5	36	30.0	0	0.0
13 - 24 months	123	27.3	16	13.0	17	13.8	35	28.4	41	33.3	2	1.6
25 - 60 months	100	22.2	20	20.0	8	8.0	35	35.0	27	27.0	4	4.0
< 60 months	367	81.6	47	12.8	46	12.5	104	28.3	104	30.0	6	1.6
6 - 10 years	54	12.0	11	20.4	0	0.0	5	9.2	2	3.7	3	5.5
11 - 15 years	29	6.4	3	10.3	0	0.0	1	3.4	1	3.4	2	6.8
6 - 15 years	83	18.4	14	16.9	0	0.0	6	7.2	3	3.6	5	6.0
Total	450	100.0	61	13.6	46	10.2	110	24.4	114	25.3	11	2.4
p			0.068		0.000		0.000		0.000		0.088	

*Note: SP: *S. pneumoniae*; HI: *H. influenzae*; MP: *M. pneumoniae*

Comment: Total 450 ARI children, the largest number of ARI children hospitalized for treatment was under 60 months old (81.6%). Children from 2 to 60 months old has the highest rate of detecting agents in RP1 and RP4 kits, gradually increasing from age of 2 months and decreasing after the over 60 months old. Influenza infection gradually increased from birth to 10 years old ($p > 0.05$). RSV infection increased from infant to 60 months, concentrated at the age of 2 - 24 months, there has no case at the age of over 60 months ($p < 0.05$). *S. pneumoniae* and *H. influenzae* infections were concentrated in the age group under 60 months old with the rate from 22.5 to 35.0%, over 60 months old, the infection rate decreased below 9.0% ($p < 0.05$). There was no child under 12 months getting *M. pneumoniae* infection, other age groups had scattered infection rates from 1.6 to 6.8% ($p > 0.05$).

The rate of pathogens were determined by RP1 and RP4 kits

Table 2. Rate of pathogens were detected by RP1 and RP4 kits

Kits	Pathogens	n	Percentage of types of viruses	Percentage
RP1	Influenzae A H3	59	13.1	13.6
	Influenzae A	1	0.2	
	Influenzae B	1	0.2	
	RSV type A	31	6.9	10.2
	RSV type B	15	3.3	
	N_p positive in RP1	107		23.8
RP1	<i>S. pneumoniae</i>	110		24.4
	<i>H. influenzae</i>	114		25.3
	<i>M. pneumoniae</i>	11		2.4
	<i>B. pertusis</i>	01		0.2
	<i>L. pneumophilla</i>	01		0.2
	<i>C. pneumoniae</i>	01		0.2
	N_p of positive in RP4	180		40.0
Total	Total of positive	242		53.8
	Total of negative	208		46.2
	Total ARI	450		100.0

Comment: The detection rate of using two kits RP1 and RP4 was 53.8% among 450 ARI inpatient children cases at Bac Giang General Hospital. Pathogens detected by RP1 kit accounted for 23.8% and RP4 kit pathogens accounted for 40.0%, respectively. The remaining 46.2% pathogens were not detected by the two kits RP1 and RP4. The rate of *H. influenzae* infection accounted for 25.3%; *S. pneumoniae* accounted for

24.4%; Influenza virus accounts for 13.6%, of which AH3 accounts for 13.1%; RSV accounts for 10.2%, of which, RSV type A accounts for 6.9%, RSV type B accounts for 3.3%; *M. pneumoniae* infection with 2.4%; *B. pertusis*, *L. pneumophilla* and *C. pneumoniae* infection each had 1 case, accounting for 0.2%.

Rate of viral - bacterial co-infection

Table 3. Rate of single infection and co-infection

Condition of infection	Single virus infection	Single bacteria infection	Co-infection	Total
Number	62	135	45	242
Percentage	13.8	30.0	10.0	53.8

Comment: In 242 ARI cases positive for pathogens of the RP1 and RP4 kits, 62 single viral infections, accounting for 13.8%, 135 single bacterial infections, accounting for 30.0%, and 45 mixed infections were detected, accounted for 10.0%.

Type of co-infection

Table 4. Types of co-infection

Types of co-infection	Number	Percentage
<i>Influenzae - S. pneumoniae</i>	6	1.3
<i>Influenzae - H. influenzae</i>	3	0.7
<i>RSV - S. pneumoniae</i>	12	2.7
<i>RSV - H. influenzae</i>	8	1.8
<i>Influenzae - S. pneumoniae - H. influenzae</i>	8	1.8
<i>RSV - S. pneumoniae - H. influenzae</i>	8	1.8
Total	45	10.0

Comment: Among 45 cases of co-infection, the highest rate of co-infection was *RSV - S. pneumoniae*, accounting for 2.7%, *RSV - H. influenzae*, *Influenza - S. pneumoniae - H. influenzae*, *RSV - S. pneumoniae - H. influenzae* all accounted for 1.8%, *Influenza - S. pneumoniae* type accounted for 1.3%, the lowest was *Influenza - H. influenzae* type accounting for 0.7% of the total ARI cases.

Seasonal prevalence of Influenza and RSV infection

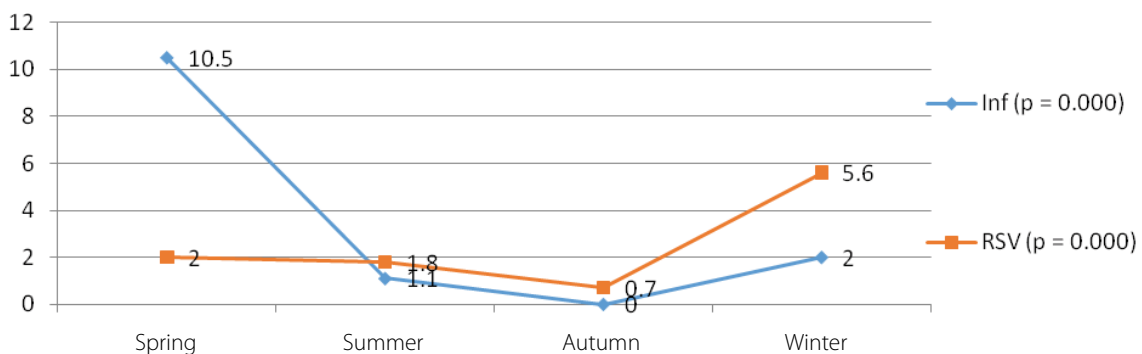


Figure 1. Seasonal *Influenza* and *RSV* infection

Comment: The rate of Influenza infection was highest in spring (10.5%); low in summer - autumn and increased gradually in winter (2.0%). *RSV* infection, highest rate in winter (5.6%), but, other seasons have scattered infection.



Rate of infection with some bacteria in months

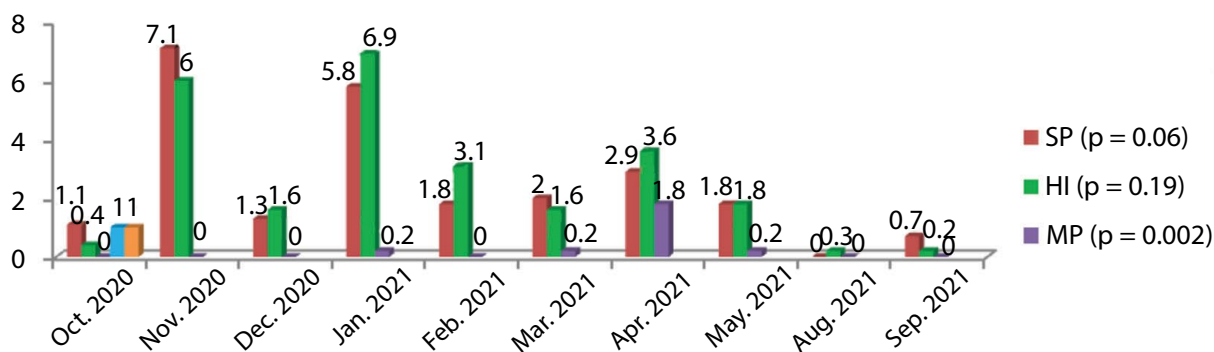


Figure 2. Rate of infection with some bacteria in months

Comment: *S. pneumoniae* infection, the highest rate was in November 2020 (7.1%) and January 2021 (5.8%), other times were sporadically infected ($p > 0.05$). *H. influenzae* infection, the highest rate were November 2020 (6.0%) and January 2021 (6.9%), with scattered infections in other months ($p > 0.05$). *M. pneumoniae* infection, the highest rate was April 2021 (1.8%), there were no case in January, March and May 2021 ($p < 0.05$).

DISCUSSIONS

Table 1 shows the age group of ARI inpatient children in Bac Giang Provincial General Hospital, the highest rate was children under 60 months accounting for 81.6%, of which, from 2 - 12 months accounted for 26.7% and under 12 months was 32.0%. Such results were lower than those Hanchi's report³, the rate of ARI children under 6 months was 54.7%. Group of age 13-24 months accounted for 27.3%, 25 - 60 months accounted for 22.2%, much higher than Hanchi's report with the corresponding results of 7.9% - 7.5%. After 5 years old, ARI children required hospitalization decreased to 18.4%, higher than Hanchi's report of 8.7%. The difference in the ARI age groups hospitalization may be due to the different disease patterns of the two hospitals. The rate of ARI due to influenza infection requiring hospitalization in our study was found in all age groups, increasing gradually from infant (8.3%) and highest in the age group of 2 - 10 years old (25 - 60 months: 20.0%, 6 - 10 years old: 20.4%), however there was no significant difference ($p > 0.05$), the trend in the proportion of ARI cases with influenza infection increased from birth to 10

years old hospitalized similar to Hanchi's report³. We observed ARI of RSV hospitalization, was seen from newborn to 60 months, the rate of RSV infection in our study was highest in infants (20.8%), ages 2 - 12 months and 13 - 24 months were 13.3% and 13.8%, 25 to 60 months was 8.0%, there was no hospitalized ARI children over 60 months in our study ($p < 0.05$), our results were similar to Hanchi's report³. There was no infection with *M. pneumoniae* in age group under 12 months, 13 - 24 months was 1.6%, infection rate gradually increased to 25 - 60 months accounted for 4.0% ($p > 0.05$), this was different from Hanchi's report with infection from newborns, over 5 years old children have *M. pneumoniae* infection rate of 6.0%, similar to Hanchi's report of 6.4%. The similarities and differences in rates of infection of *Influenza*, *RSV* and *M. pneumoniae* with Hanchi's report may be due to the disease patterns of two hospitals. In our study, the rate of *M. pneumoniae* infection in ARI children hospitalized occurred from 13 months old, however, at the time of the study, there was low number of positive cases of *M. pneumoniae*, so it may not accurately reflect the epidemiological situation of *M. pneumoniae* infection in the community, it only reflects the rate of ARI children infected with

M. pneumoniae. hospitalized in Bac Giang Provincial General Hospital. The rate of *M. pneumoniae* infection in our study was similar to Hanchi's report, however, the author's statistics show a number of *M. pneumoniae* infections in children under 1 year old that was different from our statistics. Infection with *S. pneumoniae* and *H. influenzae* can occur from infant, this rate gradually increases until 5 years old, then begins to decrease significantly. At the age of 6 years, the infection rate of these two agents was quite low, these differences were significant ($p < 0.05$).

Table 2 show that the number of positive cases by RP1 kit were 107, accounting for 23.8%, of which 13.6% was influenza and 10.2% was RSV. Our results were different from the Phung Thi Bich Thuy's report⁵, may be subjects of our study were ARI children in the Provincial Hospital, were different from children with lower respiratory tract infections at the National Children's Hospital, furthermore, Phung Thi Bich Thuy used kits to detect more pathogens such as *Parainfluenza*, *Adenovirus*, *Enterovirus*.... Maybe due to this reason, our results were lower than Weidmann et al's report (44%)⁶. The positive rate of some pathogens in RP1 kit, including influenza AH3, was 13.1%, higher than Hanchi's report (5.4%). There was only 01 case of influenza type B and 01 case of influenza type A with no type identified (0.2%). The positive rate of RSV was 10.2%, lower than Hanchi's report of 23.2%³, the different rate of influenza and RSV infection in our study and Hanchi's report may be due to different disease patterns at the two study sites. During the research period, no cases of AH1N1 influenza infection were detected. It is possible that AH3 influenza began to appear in Europe in 2020 and gradually replaced AH1N1 influenza in Europe and around the world⁷⁻¹¹. The results of this study show that in Bac Giang, by October 2020, the influenza AH3 had completely replaced the influenza AH1N1 and created a wave of influenza epidemics.

The bacteria that cause ARI in children are complex, consisting of pathogens easy to culture

such as *S. aureus* to fastidious pathogens such as *H. influenzae* or bacteria that cannot be cultured with conventional culture media such as *M. pneumoniae*, *C. pneumoniae*, *L. pneumophila*... The Seegene manufacture has developed RP4 kit enabled to detect some common agents but difficult to culture that cause respiratory infection. The results of our study in Table 2 show that in 450 ARI children, 180 cases (40%) were infected with at least one of the bacteria that were difficult to culture in the list of RP4 kit. The infection rate in our study was much higher than Phung Thi Bich Thuy's report (15%)⁵. The proportion of bacteria detected: *H. influenzae* (25.3%) of ARI cases, *S. pneumoniae* (24.4%). The rate of *M. pneumoniae* infection was (2.4%), this was similar to Hanchi's report (2.6%)³, but, lower than Kutty's report (8.0%)¹², although this is a small percentage, the detection of *M. pneumoniae* was very important, because it is no wall bacteria, develops inside the host cells and could not culture by conventional culture media, it must be determined by other methods such as antigen or antibody tests. Real-time PCR is a more sensitive method of detecting *M. pneumoniae* than the antigen test, reported by Chien and CS¹³. Without real-time PCR detection, it will be difficult for labos to detect *M. pneumoniae* infection. There was 1 case of *B. pertussis* detected, this agent was difficult to culture, recently, there is vaccine, according to Muloiwa¹⁴, from the 2000s onwards, the incidence of *B. pertussis* in the developing countries, the average input is 5 - 27% when detect by real-time PCR. Although *B. pertussis* infection is rare at the present, the detection of of this agent is meaningful in diagnosis, treatment of whooping cough, and in epidemiological investigations. We detected 01 case of *L. pneumophilla*, this was also a rare and difficult to culture, mainly causing nosocomial infections. There was little reports of *L. pneumophila* infection in Vietnam and in the world, only the report of Andersen (1981)¹⁵ screening by serological tests from 1972 - 1974 in children diagnosed with ARI were 5.8% of 52 children at two units of pediatric



treatment in Denver and Atlanta (USA). Because this was not common pathogens, many laboratories do not have the ability to detect it. The development of a kit to detect *L. pneumophilla*, which was very important in diagnosing the cause, which was an effective and timely for the labos and the clinicians. Combining two kits RP1 and RP4 helped to detect the cause of ARI for 53.8% in 450 ARI children, this result was lower than Hanchi's report with 72.5% positive with at least one pathogen³, the differences may due to the list of pathogens detected in our study were different from the list of pathogens in Hanchi's study, we identified more bacteria and Hanchi detected more viruses.

Table 3 shows the prevalence of co-infection accounting 10.0% of ARI cases. According to DeMuri¹⁶, when getting a viral infection, it will cause better conditions for bacteria to grow and invade the host defence. The types of co-infection were shown in Table 4, in which the highest rate of co-infection was RSV - *S. pneumoniae* (2.7%). The rate of viral co-infection with two bacteria including influenza - *S. pneumoniae* - *H. influenzae* and RSV - *S. pneumoniae* - *H. influenzae* both accounted for 1.8%. These co-infection rates show that the random combinations. Determining the rate and type of co-infection helps clinicians consider the use of antibiotics for the empiric antibiotics therapy.

There were many seasonal pathogens caused ARI, especially viral infections. Our result was in figure 1. The rate of influenza infection increased in the spring with 10.5% of ARI children admitted to hospital. However, in 2020 and 2021, the COVID-19 pandemic occurred. At this time, Bac Giang has applied COVID-19 prevention strictly, so that, it helped to prevent other respiratory viruses, and shortened the winter-spring flu epidemic in late 2020 - early 2021, it was different from Hanchi's report with the highest influenza infection rate in winter (13.0%)³. The results in figure 1 also showed that the RSV infection rate increases highest in winter with 5.6% of ARI children, similar to the reports of Hanchi and Phung Thi Bich Thuy^{3,5}.

Figure 2 showed the infection rate of some bacteria in months. The infection rate increases in the months of winter and spring. However, the infection of *S. pneumoniae* and *H. influenzae* did not have a significant seasonal feature ($p > 0.05$). *M. pneumoniae* infection raised in the spring - summer from January to May. The seasonal feature of *M. pneumoniae* infection was significant ($p < 0.05$).

CONCLUSIONS

Among 450 ARI children, 23.8% were positive with at least one pathogens using RP1 kit, of which 13.6% were influenza, and 10.2% RSV. There were 40.0% positive with at least 1 pathogen using the RP4 kit, including 24.4% *S. pneumoniae*, 25.3% *H. influenzae*, 2.4% *M. pneumoniae*, 0.2% *B. pertusis*, 0.2% *L. pneumophila*, and 0.2% *C. pneumoniae*. Combining two kits of RP1 and RP4, the prevalence of positive and neagative was 53.8% and 46.2%, respectively; and the rate of positive with at least one kind of virus, one kind of bacteria, and co-infection was 13.8%, 30.0%, and 10.0%, respvatively. The co-infection patterns still remain unclear and could be a result of random combination. Influenza, RSV and *M. pneumoniae* infections were significant affected by seasoning, while *S. pneumoniae* and *H. influenzae* infections were sporadic all over the time.

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