Medical Laboratory Technology Journal
9(1), 2023, 53-62
Received 2023-04-04; Revised 2023-24-04; Accepted 2023-02-05
Available online at : http://ejurnal-analiskesehatan.web.id

Identification of Acute Respiratory Infection Patients Using RP2 Nested Multiplex PCR Test in Jakarta, Indonesia

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DOI: 10.31964/mltj.v8i2.519

Abstract: Acute Respiratory Infection (ARI) is an acute respiratory disease caused by infectious organisms transmitted between humans. Viruses and bacteria generally cause the cause of ARI infection. Other viruses that can also cause ARI are Influenza, Adenovirus, Enterovirus, and Respiratory Syncytial Virus. This study aims to determine the causes of bacterial or viral ARI infection with RP2 Nested Multiplex RT-PCR. The research methodology is cross-sectional. The sample used was 50 people with purposive sampling technique in patients with ARI who examined bacteria and viruses using RP2 Nested Multiplex RT-PCR. The research was located at the Laboratory of the United States Embassy in Jakarta, Indonesia. The results showed that 26 (52%) men suffered more from ARI patients than 24 women (46%), with the most age group being children, 28 people (56%). The three symptoms that many patients in this study felt were that 40 patients (80%) experienced nasal congestion, 38 patients (76%) experienced fever, and 32 patients (64%) coughed. The results of the organisms in the RP2 nested multiplex RT-PCR examination showed that 100% of the causes of ARI were viruses with the highest prevalence (40%) originating from the Human Rhinovirus/Enterovirus. The reason for ARI in this research is from a virus (100%), so antibiotics are not needed for this patient.
Keywords: Acute respiratory infection; bacteria; RP2 nested multiplex; virus

INTRODUCTION

Acute Respiratory Infection (ARI) is a disease with mild to severe manifestations with symptoms including fever, cough, and often sore throat, runny nose, shortness of breath, wheezing, or difficulty breathing (Hassen et al., 2020). ARI is a significant health problem because it causes high infant and child mortality, 1 in 4 deaths yearly. In 2016 WHO stated that the incidence of acute respiratory infection (ARI) in toddlers at the world level was between 15-20%, the incidence of ARI in developing countries was 0.29% and in industrial areas 0.05%, while the incidence of ARI in Indonesia 151 million people per year (Shi et al., 2020). Meanwhile, the prevalence of ARI in Indonesia is symptomatic at 9.3% and pneumonia at 4.0% (Ministry of Health of Republic Indonesia, 2018).

Droplets generally transmit ARI with symptoms such as sore throat, nasal congestion, coughing, sneezing, rhinorrhea, headaches, watery eyes, body aches, and fever (Lee et al., 2019). Symptoms of ARI can last up to 14 days, but giving antibiotics will not shorten the recovery of the disease (ECDC, 2022). Viruses and bacteria generally cause the cause of ARI infection. One of the most infective is Rhinovirus. Other viruses that can also cause ARI are Influenza, Adenovirus, Enterovirus, and Respiratory Syncytial Virus (Fitzner et al., 2018).

The diagnosis of ARI in Indonesia is usually based on the symptoms that arise rather than from a diagnostic point of view (Surury & Azizah, 2022). Whereas in

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developed countries, the diagnosis of ARI is by examining the patient's respiratory tract fluids and lung swelling by listening to abnormal sounds when the patient with ARI breathes. Then after a suspected infection of the respiratory tract, the doctor will take a swab from the nose or mouth, sputum, or respiratory tract rinses. The patient will provide a sputum sample to check the type of virus or bacteria that causes the disease (Hanson et al., 2020). Diagnostic examinations or laboratory tests in patients with ARI in these samples can be carried out by culture examination, Rapid Diagnostic Test (RDT), and PCR (Noviello & Huang, 2019). Diagnostic examination using PCR performed on patients with ARI using RP2 Nested Multiplex RT-PCR can make a diagnosis faster (± 45 minutes) and has a specificity of 99.3% (Creager et al., 2020).

The results found from this research which conducted from three geographically distinct from USA (Colombus, Maywood, and Salt Lake City) using Nasopharyngeal Swab specimens and RP2 Nested Multiplex RT-PCR (BioMeriux), were 31.1% Human Rhinovirus/ Enterovirus, 12.3% Respiratory Syncytial Virus, 7.3% Adenovirus (7.3%), 5% Influenza A, and 5.9% Coronavirus (CoV-229E, -HKU1, -NL63, and -OC43). The number of organisms that can be detected can be influenced by the population of the study, where during the study period, there was no spread of the MERS-CoV and FluA H1 viruses. (Amy L. Leber et al., 2018). It was also found in another research using retrospective analysis from patients with ARI hospitalized at Chaim Sheba Medical Center in Israel. This research used Nasopharyngeal Swab specimens and NucliSENS easyMAG (BioMerieux), with the results being 28% Human rhinovirus (hRV), 28% adenovirus, and 26% Respiratory Syncytial Virus (RSV), while influenza viruses, parainfluenza-3 and hMPV were the least common (< 10%) (Pomeranz et al., 2019). The research on acute respiratory infections conducted in geographically distinct locations with molecular laboratory tests yielded similar results, with the majority of ARI originating from viruses, namely Human Rhinovirus (hRV), Adenovirus, Respiratory Syncytial Virus (RSV), and influenza viruses.

Research on Acute Respiratory Infection (ARI) in Indonesia is only in case studies, the causative factors come from the environment, and the sample coverage is only in children. In Indonesia, ISPA ranks first as the cause of death in the infant and toddler group, with a percentage of 38.80% of all under-five deaths with the distribution of research data obtained from Bali - Indonesia, namely 40-60% at visits to health centres and 15-30% at stops in the hospital (Oktarina et al., 2020). The distribution of ISPA cases in the last two years (2018-2019) in DKI Jakarta Province shows a fluctuating increase due to poor air quality, which is based on the Air Pollution Standard Index (PSI) with a PM10 value for Kelapa Gading and Cipayung Districts (Surury & Azizah, 2022). In addition to the PSI factor, other factors are also related, namely lifestyle and household environmental factors such as the use of dirty fuel, the presence of smokers in the household, poor quality drinking water, and low availability of toilet facilities (Lutpiatina et al., 2022). As a measure of controlling ARI in Indonesia, the authors wanted to know the causes of Acute Respiratory Infection (ARI) from the patients who were suspected of having ARI by carrying out laboratory tests by taking samples from nasopharyngeal swabs to find out the microorganisms that cause ARI using RP2 Nested Multiplex RT-PCR. It can be used as data about the organisms that cause ARI, which was conducted in Indonesia, so the correct diagnosis follows Indonesian patients' characteristics. It can be considered for clinical doctors to provide the proper treatment for this patient.

MATERIALS AND METHODS

Research Design

This research has received ethical approval from the ethical committee of the Budhi Asih Jakarta Hospital with number: 246/KEP-ETIK/V/2021. This research is a descriptive analysis with a cross-sectional approach using secondary data from the RP2 nested multiplex RT-PCR examination results in ARI patients.

Population and sample research

The sample used in this research were ARI patients from August 2018 to May 2021, taken using a purposive sampling technique according to the criteria. The criteria used in this study were based on inclusion and exclusion criteria. The inclusion criteria were ARI patients of all ages and genders, patients experiencing one or more clinical symptoms (fever, chills, headache, runny/runny nose, sore throat, cough, muscle/body aches and fatigue), and patients suffering from ARI—less than 14 days. Meanwhile, the exclusion criteria were patients who had ARI for more than 14 days and had no clinical symptoms leading to ARI. The sample size was 50 suspected ARI examined for bacteria and viruses using RP2 Nested Multiplex RT-PCR. The sample of this research is the facilitation of diplomats suspected of suffering from ARI, which was carried out at the Clinical Laboratory of the United States Embassy in Jakarta, Indonesia.

Materials and Research Tools

The materials used in this study were Respiratory Panel 2 (RP2 reagents). The materials available in the kit were RP2 pouches, sample buffers, hydration injection vials, sample injection vials, and sterile transfer pipettes. The tool used in this research is BioFire FilmArray Respiratory Panel 2.0 (BioFire Diagnostics, Inc.). FARP is a multiplex polymerase chain reaction technique that can detect 14 viral parameters and four bacterial parameters. The principles used during the inspection process using this tool are lysing the sample with agitation using beads, extracting to purify all the nucleic acids in the sample using magnetic bead technology, performing nested multiplex PCR, and melting endpoint curve data used in detecting results for each target, on the RP2 Filmarray series.

Collection / Research Procedure

The procedure in this research was carried out based on (BioNumerics, 2021) by having three stages of the procedure, there are:

Preparation of Pouch Filmarray RP2

Clean the work table and Filmarray pouch loading station with a 10% bleach solution, followed by a water rinse. Remove the Filmarray RP2 pouch from the vacuum packaging and insert the Filmarray RP2 pouch by aligning the red and blue labels according to the arrows into the Filmarray pouch loading station. For sample vials (red caps), put them directly into the Filmarray pouch loading station. As for the Hydration Injection Vial (blue cap), put it closed in the FilmArray Pouch Loading Station, insert the tip of the needle into the hydration port pouch, press the modifier until you hear a weak popping sound, and wait until the volume of the hydration liquid is just right to be drawn into in a pouch with a vacuum system, and make sure the pouch is hydrated with the seal being perforated.

Sample mixture preparation

Add the sample buffer into the injection vial by slowly dispensing with full pressure. Homogenize the specimen (nasopharyngeal swab) by vortexing. I am adding the specimen to the sample buffer in the sample injection vial with the transfer pipette provided up to the third line. Close the injection vial sample tightly, homogenize three times, and place it into the FilmArray Pouch Loading Station.

Pouch Filmarray is reading RP2.

Lift the injection vial sample (red cap) by rotating slowly, leaving the cap behind, insert the tip of the needle into the sample port pouch, which is under the red arrow on the FilmArray Pouch Loading Station, and press the modifier until you hear a weak popping sound, wait until the volume of The specimen mixture is drawn into the pouch by a vacuum system. Inspect by following the processing steps on the computer screen from the Filmarray 2.0 tool, from placing the Filmarray R2 pouch on the tool until pressing the “Start Run” button. When the inspection starts, the computer screen will display a list of the steps to be performed by the tool and the remaining time required for the inspection to be completed. 8. When the inspection is complete, follow the instructions on the computer screen to dispose of the pouch into the biohazard container, and the inspection file is automatically saved in the Filmarray 2.0 database.

Data Analysis

All data obtained were processed using the IBM SPSS Statistics 26 program in univariate and bivariate descriptive analysis. Data on ARI sufferers by sex and age were analyzed univariately in a distribution table. Meanwhile, symptom data and multiplex nested RP2 RT-PCR examination results were processed using “Multiple Response” analysis which is presented as a percentage table.

RESULTS AND DISCUSSION

After collecting data at the Clinical Laboratory of the United States Embassy, 50 patient data with ARI were obtained along with clinical symptoms and RP2 nested multiplex RT-PCR examination results from August 2018 to May 2021. The collected data were subjected to descriptive analysis to determine the characteristics of ARI patients and “Multiple Response” analysis of clinical symptoms of ARI sufferers and RP2 nested multiplex RT-PCR test results. The data is displayed in tabular form as follows Table 1.

Table 1. Data Distribution of ARI suffered by Sex and Age

Age Group	Gender		Total	
	Man	Woman	N	%
Children	15	13	28	56
Mature	10	10	20	40
Elderly	1	1	2	4
Total number	**26**	**24**	**50**	**100**

Based on the age grouping of children (0-18 years), adults (19-60 years) and elderly (> 60 years) in Table 1, it shows that ARI sufferers are more common in the age group of children as many as 15 people (%) with male gender and as many as 13 people (%) with the female gender. The adult age group was 20 people (%) with the same percentage of the male and female sex, while the elderly age group was two people (%) with the same percentage of the male and female sex.

Acute respiratory infection (ARI) is an infection that affects the sinuses, throat, airways or lungs. In this study, more men suffered from ARI, with a percentage of 52% (26 people) and 48% women (24 people). ARI is possibly caused by differences in occupation, lifestyle, exposure, level of vulnerability, and use of health facilities such as primary health services, which are visited by more women and children than men, so the number of recorded diseases is likely to differ according to type male and female genitalia (Yunus *et al.*, 2020). For the age criteria of ARI patients in this study, the

youngest was one year old, while the oldest was 62. Of these ages, we grouped them into three categories based on the Law of the Republic of Indonesia number 13 of 1998, so that 28 people (56%) were children, 20 people (40%) were adults, and two people (4%) were elderly. ARI patients, especially those with upper respiratory tract infections, significantly cause morbidity and mortality worldwide. However, ARI is usually more severe for children, older people, people with immune deficiencies, and all individuals from all populations and age groups who are susceptible (Yanagihara, 2019). Infants and toddlers are most vulnerable to ARI because their immune systems are still weak and immature, so they are more at risk of exposure to ARI (Subramony et al., 2016).

Table 2. The Results of Symptoms in ARI Patients Use Multiple Response Analysis

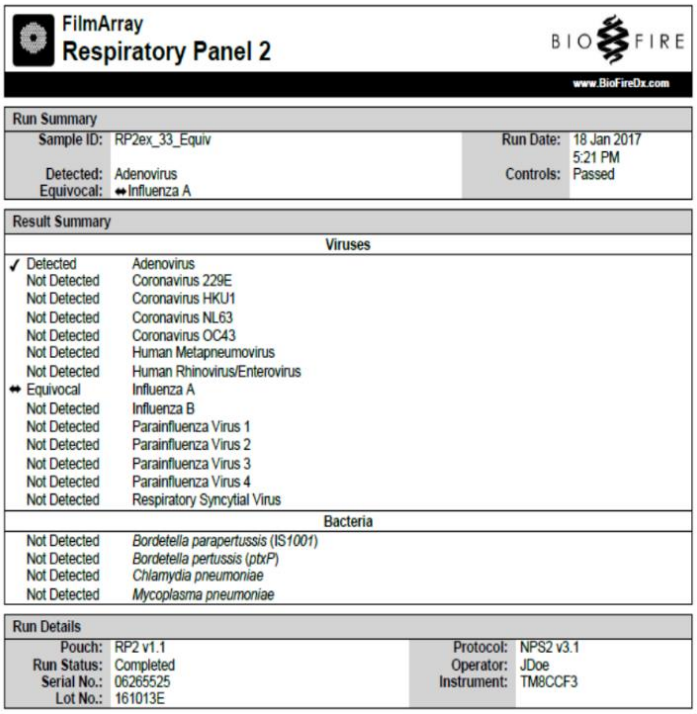
Type and Subtype Microorganism	N	Percent (%)	Percent of Cases (%)
Fever	38	18,3	76
Chills	14	6,7	28
Headaches	17	8,2	34
Nasal congestion	40	19,2	80
Sore throats	24	11.5	48
Cough	32	15,4	64
Muscle ache	17	8,2	34
Tired	26	12.5	52
Total	**208**	**100**	**416**

Based on table 2 shows that 40 people (80%) complained of nasal congestion, 38 people (76%) complained of fever, 14 people (28%) felt chills, 17 people (34%) had headaches, 40 people (80%) found rhinorrhea/nasal congestion, 24 people (48%) had a sore throat, 32 people (64%) had a cough, 17 people (34%) had complaints of muscle/body pain, and 26 people (26%) felt tired.

Table 3. The Results of Types and Subtypes of Microorganisms as a Result of RP2 Nested Multiplex RT-PCR Test Using Multiple Response Analysis

Type and Subtype Microorganism	N	Percent (%)	Percent of Cases (%)
Adenoviruses	3	5,7	6.0%
Coronavirus HKU1	2	3,8	4.0%
Coronavirus NL63	2	3,8	4.0%
OC43 coronavirus	1	1,9	2.0%
Human Metapneumovirus	4	7,5	8.0%
Human Rhinovirus/Enterovirus	20	37,7	40.0%
Influenza A H1-2009	2	3,8	4.0%
Influenza A H3	5	9,4	10.0%
Parainfluenza Virus 1	1	1,9	2.0%
Parainfluenza Virus 2	1	1,9	2.0%
Parainfluenza Virus 3	4	7,5	8.0%
Parainfluenza Virus 4	1	1,9	2.0%
Respiratory Syncytial Virus	7	13,2	14.0%
Total	**53**	**100**	**416**

Acute respiratory infections (ARI) can cause a symptom burden with several combinations of nasal congestion, rhinorrhea, sore throat, and cough (Kardos et al., 2020). The symptoms caused in this study sample were 40 patients (80%) had a runny nose (rhinorrhea) or nasal congestion, 38 patients (76%) had fever, 32 patients (64%) coughed, and 26 patients (12.5%) experienced fatigue. Twenty-four patients (11.5%) had sore throats, 17 patients (8.2%) had headaches, and 17 patients (8.2%) had muscle/body pain. This is in line with the results of the study (Lee et al., 2019), where the symptoms complained of by the patients in this study, such as runny (rhinorrhea), nasal congestion, sneezing, and mucus production, are signs and symptoms resulting from inflammation of the mucous membranes in the upper respiratory tract. Other symptoms that can be caused are fever, tiredness, headache, swallowing pain, and wheezing (Green et al., 2016).



FilmArray
Respiratory Panel 2

BIOFIRE
www.BioFireDx.com

Run Summary

Sample ID:	RP2ex_33_Equiv	Run Date:	18 Jan 2017 5:21 PM
Detected:	Adenovirus	Controls:	Passed
Equivocal:	⬌Influenza A		

Result Summary

	Viruses
✓ Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
⬌ Equivocal	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus
	Bacteria
Not Detected	*Bordetella parapertussis* (IS*1001*)
Not Detected	*Bordetella pertussis* (*ptxP*)
Not Detected	*Chlamydia pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

Run Details

Pouch:	RP2 v1.1	Protocol:	NPS2 v3.1
Run Status:	Completed	Operator:	JDoe
Serial No.:	06265525	Instrument:	TM8CCF3
Lot No.:	161013E		

Figure 1. Filmarray RP2 Examination Results for Each Sample

The type and subtype of RP2 nested multiplex RT-PCR examination results in Table 3 and Figure 1 shows that three people (6.0%) were infected with Adenovirus, two people (4.0%) Coronavirus HKU1, two people (4.0%) Coronavirus NL63, one patient (2.0%) Coronavirus OC43, four patients (8.0%) Human Metapneumovirus, 20 patients (40.0%) Human Rhinovirus/Enterovirus, 2 patients (4.0%) Influenza A H1-2009, five patients (10%) Influenza A H3, one patient (2.0%) Parainfluenza Virus 1, 4 patients (8.0%) Parainfluenza Virus 2, 1 patient (2.0%) Parainfluenza Virus 3, and 7 patients (14.0%) Respiratory Syncytial Virus. Based on the type and subtype of microorganisms, the RP2 nested multiplex RT-PCR examination results, all patients with ARI were caused by a viral infection. The percentage of the emergence of viral infection was as much as 106% of 50 ARI sufferers. Some patients with ARI are infected by more than 1 type/ subtype of the virus.

In identifying the organisms that cause ARI in this study, RP2 nested multiplex RT PCR was used using BioFire FilmArray Respiratory Panel 2.0. The inspection technique through PCR is a preferred and acceptable molecular approach in detecting viruses that cause ARI because of its automation and work with large samples

(Subramony et al., 2016). FilmArray RP2 is a test instrument that has worked in the US since November 2017 and includes tests in the Microbiology checklist. This test evaluates the compatibility of nucleic acids with currently circulating microbial strains. FilmArray RP2, part of the fourth generation of PCR multiplex panels since its introduction in 2011, provides updates on primers based on a re-examination of known nucleic acid sequences for most pathogens and adjustment of assay conditions to maximize yield (Amy L. Leber *et al.*, 2018). In the present study, viruses are a common cause of upper respiratory tract infection in the adult and pediatric populations, as seen in our study group. Virus detection was higher than bacterial targets using the RP2 FilmArray, which showed an increased positive detection rate for all virus targets when examining ARI symptoms (Heinonen et al., 2016).

The various symptoms of ARI are caused by more than 200 types of viruses, with 50% originating from rhinoviruses with at least 99 strains and being pathogenic (Kardos et al., 2020). The examination results of this study showed that 50 people were infected with 1 type of virus, and three people were infected with more than 1 type of virus, with the highest prevalence of 40% (20 people) being infected with the Human Rhinovirus/ Enterovirus.

Human rhinovirus (hRV) is one of the most common causes of acute respiratory disease (ARI), which involves the upper and lower respiratory tract (LRT). The hRV virus is a single-stranded, non-enveloped RNA virus belonging to the Picornaviridae family with three species and 150 serotypes (Tran et al., 2016). Transmission of the hRV virus occurs to fellow humans both directly and through contaminated media with clinical symptoms similar to mild flu-like illness to severe respiratory disease. It even requires intensive care unit (ICU) care (Drysdale et al., 2017). hRV infection affects all age groups, at a rate of 2-3 times per year in adults and at a higher rate of up to 12 illnesses per year in children (Pomeranz et al., 2019). With the increased implementation of clinical laboratory tests for the molecular field, the Human Rhinovirus can be fascinating to be diagnosed as a pathogen causing ARI, especially in infants and elderly patients (Heinonen et al., 2016). Positive RV detection was found in 58% of patients with ARRI under the age of 5 years, as many as 243 people, of which 16% were Rhinovirus type A (Pomeranz et al., 2019).

The number of bacteria detected was relatively low with the RP2 FilmArray—the difference in the actual prevalence of the disease during the study period. Simultaneously detecting viruses and bacteria in ARI using Nasopharyngeal swab specimens is less than optimal in lower respiratory tract infections (Kakuya et al., 2017) because the cause of ARI infection in this study was 100% viral, so antibiotics were not needed to treat the disease. Treatment for ARI infections aims to relieve symptoms with decongestant drugs and antihistamines to reduce cough symptoms, respiratory tract obstruction and other symptoms in adults (Kardos et al., 2020). Treatment through administering antiviral drugs in the early stages of infection can shorten symptoms and duration of hospitalization and reduce the risk of complications. However, using antibiotics does not improve symptoms, can cause side effects and contribute to antibiotic resistance (Lee et al., 2019).

There are two kinds of limitations to this research. The first is that the sample was conducted from one geographically isolated from Indonesia, Jakarta. The choice of one geographical district was based on a laboratory with a FilmArray RP2 Nested PCR tool currently available at the Clinical Laboratory of the United States Embassy in Jakarta, Indonesia. In addition, when the research was conducted during the COVID-19 pandemic, there were restrictions in all respects. The second is the use of FilmArray RP2 Nested PCR. FilmArray RP2 is a qualitative examination with either

Positive (Detectable) or Negative (Not Detected) results. The qualitative examination serves as an initial screening so that it cannot detect viral load in ARI infection. FilmArray RP2 Nested PCR tool that can be utilized in detecting various kinds of microorganisms (bacteria or viruses) that cause Acute Respiratory Infection (ARI). When the correct diagnosis is made in a patient with ARI, it can be taken into consideration for clinical doctors to provide the proper treatment for this patient. Following the results of this study, the causative microorganism originates from a virus (100%), so administering antiviral drugs can shorten symptoms and duration of hospitalization and reduce the risk of complications. Meanwhile, antibiotics do not support the treatment of ARI because antibiotics do not improve symptoms or shorten the course of the disease.

CONCLUSION

The RP2 nested multiplex RT-PCR examination results in this study showed that 100% of the causes of ARI were viruses, with the highest prevalence (40%) originating from the Human Rhinovirus/Enterovirus. Because the cause of ARI is derived from a virus, antibiotics are not needed for sufferers of ARI.

ACKNOWLEDGEMENT

Not applicable

CONFLICT OF INTEREST

There are no conflicts of interest.

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