

Respiratory viruses detection using the FilmArray Panel

Deteksi Virus Penyebab Infeksi Saluran Napas menggunakan FilmArray Panel

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ABSTRACT

Background: Respiratory infections, including pneumonia, are a leading cause of global mortality. These infections can be caused by various pathogens, including typical and atypical bacteria, as well as viruses, fungi, and parasites. Rapid and accurate diagnosis is crucial, especially in cases requiring intensive care, to ensure timely and appropriate treatment.

Objective: This research aimed to obtain an overview of the application of the FilmArray method, a nested multiplex PCR base method, in determining the diagnosis and management of respiratory tract infections.

Methods: This study was a cross-sectional study with a descriptive analytic design on patients with respiratory tract infections. The study was conducted from April to June 2022 at the Biomolecular Laboratory, Laboratory Installation of RSPI Prof. Dr. Suliarti Saroso.

Results: Of the 72 patients examined, 27 (37.5%) tested positive for one of the viruses, while 45 (62.5%) tested positive for neither viruses nor bacteria. Among the positive patients, the most commonly detected virus was human rhinovirus/enterovirus (48%), followed by SARS-CoV-2 (48%), and human metapneumovirus (4%). No atypical bacteria or a double infection of virus and bacteria were detected.

Conclusion: This research indicates that molecular-based methods using the FilmArray respiratory panel can be used for the rapid and accurate detection of respiratory tract infection cases, especially those caused by respiratory viruses and atypical bacteria.

Keywords: acute respiratory infection, FilmArray, multiplex assay, pathogen detection

ABSTRAK

Latar Belakang: Infeksi saluran napas, termasuk pneumonia merupakan penyebab utama kematian global. Infeksi ini dapat disebabkan oleh berbagai patogen, termasuk bakteri tipikal dan atipikal, serta virus, jamur, dan parasit. Diagnosis yang cepat dan akurat sangat penting, terutama pada kasus dengan perawatan intensif, untuk memastikan pengobatan yang tepat waktu dan sesuai.

Tujuan: Penelitian ini bertujuan mendapatkan gambaran penggunaan metode pemeriksaan FilmArray yaitu suatu metode berbasis multipleks PCR secara nested, dalam penegakkan diagnosis dan tatalaksana infeksi saluran napas. Studi ini merupakan studi potong lintang dengan desain studi deskriptif analitik terhadap pasien infeksi saluran pernafasan. Studi dilaksanakan pada bulan April sampai Juni 2022, di Laboratorium Biomolekuler, Instalasi Laboratorium RSPI Prof. Dr. Suliarti Saroso.

Hasil: Dari 72 pasien yang diperiksa, sebanyak 27 pasien (37,5%) menunjukkan hasil positif terhadap salah satu virus, sedangkan 45 pasien (62,5%) tidak terdeteksi adanya

virus maupun bakteri. Di antara pasien yang positif, virus yang paling banyak terdeteksi adalah Human rhinovirus/enterovirus (48%), diikuti oleh SARS-CoV-2 (48%), dan Human Metapneumovirus (4%). Tidak ditemukan adanya bakteri atipik maupun infeksi ganda virus dan bakteri.

Kesimpulan: Dari penelitian ini didapatkan bahwa metode berbasis molekular dengan menggunakan FilmArray panel respiratori dapat digunakan untuk mendeteksi secara cepat dan akurat kasus-kasus infeksi saluran nafas, terutama yang disebabkan oleh virus-virus respirasi dan bakteri atipikal.

Kata kunci: deteksi patogen, FilmArray, Infeksi Saluran Nafas Akut (ISPA), multiplex assay

INTRODUCTION

According to the World Health Organization (WHO), respiratory tract diseases including pneumonia and influenza are among the leading causes of death in the world.¹ Based on the Global Burden of Disease, Injuries, and Risk Factors Study (GBD) report in 2016, the number of deaths due to lower respiratory tract infections such as pneumonia or bronchiolitis was estimated at 2.3 million deaths.² GBD report in 2019, estimated deaths due to upper respiratory tract infections were around 14,9137 cases.³ In Southeast Asia, East Asia, and Oceania, 431,000 deaths were reported in 2021 due to respiratory infections such as pneumonia.⁴

Acute respiratory tract infections (ARI) remain a major health problem in developing countries, including Indonesia. Data on respiratory tract infections is scarce. Pneumonia is reported to be the leading cause of death globally in children, while pneumonia ranks sixth among adults.⁵ Based on national data from 2018, lower respiratory tract infections were the third leading cause of infant mortality and the second leading cause of disability in Indonesia.⁶

There are several pathogens that cause respiratory tract infections, namely bacteria (typical and atypical) and non-bacteria such as viruses, fungi and parasites.⁶ Based on a study, in pneumonia, polymicrobial infections are often found, namely non-bacterial pathogens such as viruses are also found.⁷ Typical bacteria as the cause of respiratory tract infections are detected using culture methods as the gold standard for diagnosis, but this is different from atypical bacteria and viruses, both of which are difficult to culture and even using serology or antigens are often not detected, so there is often a delay in diagnosis.⁷ Speed and accuracy of diagnosis are crucial, especially in intensive care patients, to ensure appropriate and prompt treatment. Detection using molecular methods offers an alternative test for diagnosing atypical or difficult-to-culture pathogens.⁷

In cases with infection Polymicrobial, multiplex molecular examination method is also an alternative examination where this method can detect several pathogens simultaneously so that it can shorten the diagnosis time.⁷ One of the technologies in the molecular method is FilmArray, where this technology can be used to detect several pathogens simultaneously.⁸

The use of FilmArray panels for patient diagnosis has been carried out to detect and identify several pathogens from throat swab specimens⁸ and regarding the impact of the use of FilmArray respiratory panels in patients who come to the emergency room with complaints of respiratory tract infections on the length of time for test results to be released.⁹ The results of both studies showed that test results can be reported in <4 hours, which certainly helps speed up patient diagnosis so that patients receive therapy quickly.^{8,9}

Based on the data presented above, the research team is interested in conducting research using a FilmArray respiratory panel to detect respiratory pathogens in inpatients at Sulianti Saroso Infectious Diseases Hospital (RSPI Sulianti Saroso). This study aims to provide an overview of the use of the FilmArray examination method in diagnosing and managing respiratory tract infections.

METHODS

Study design

This study was a cross-sectional study with a descriptive analytical design in patients with respiratory tract infections. The study was conducted from April to June 2022 at the Biomolecular Laboratory and Laboratory Installation of Prof. Dr. Sulianti Saroso Infectious Diseases Hospital.

Data source and sampling procedure

The study population was adult patients with respiratory tract infections who were hospitalized at Prof. Dr. Sulianti Saroso Infectious Diseases Hospital. The sample used was all patients who met the inclusion criteria, namely age >18 years and diagnosed with pneumonia.

Data collection

Nasopharyngeal swab specimen and The oropharynx was examined for SARS-CoV2 using the qRT-PCR examination technique in accordance with the screening guidelines for patients receiving respiratory tract infections and then continued with examination using the Biofire FilmArray.®RP 2.1plus respiratory panel. FilmArray is a nested polymerase chain reaction (PCR)-based method developed by bioMerieux, France.

Measurement and instruments

This study used Biofire®respiratory panel RP 2.1plus, targeting 19 types of viruses, namely *Adenovirus*, *Coronavirus 229E*, *Coronavirus HKU1*, *Coronavirus NL63*, *Coronavirus OC43*, *Middle East respiratory syndrome coronavirus* (MERS-CoV), *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2), *Human metapneumovirus* (HMPV), *Human rhinovirus/enterovirus* (HMPV), *Influenza A virus*, *Influenza A virus A/H1*, *Influenza A A/H3 virus*, *Influenza A virus A/H1-2009*, *Influenza B virus*, *Parainfluenza virus 1*, *Parainfluenza virus 2*, *Parainfluenza virus 3*, *Parainfluenza virus 4*, *Respiratory syncytial virus* and 4 atypical bacteria, namely *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumonia* and *Mycoplasma pneumonia*.¹⁰ The film array manufacturing process is carried out according to the manufacturer's instructions. Biofire®The RP 2.1plus respiratory panel can optimally detect up to three microorganisms from the available detection panel with a limit of detection (LOD) of 165 genome copies/ml.^{11,12}

Data analysis

The results of the examination will be analyzed descriptively.

Ethical considerations

This study has received ethical approval from the Ethics Committee of Prof. Dr. Sulianti Saroso Infectious Diseases Hospital (RSPI) under ethics approval number 21/XXXVIII.10/IV/2022.

RESULTS

A total of 72 inpatients who met the inclusion criteria were included in the study. The demographic and clinical characteristics of the 72 subjects (Table 1). In Table 1, based on sample characteristics, the majority were in the productive age group (18-64 years) as many as 57 people (79%), gender did not differ between men and women, although there were more men with a total of 37 people (51%), the treatment room for most samples was treated in a non-ICU room as many as 54 people (75%). Table 1 also

shows that the radiology results of the samples upon admission were mostly pneumonia as many as 31 people (43%), and the results of the COVID-19 PCR screening were mostly negative with a total of 59 people (82%).

Table 1. Sample characteristics

Characteristics	N (%)
Age	
- productive (18-64 years)	57 (79)
- non-productive (>64 years)	15 (21)
Gender	
- Man	37 (51)
- Woman	35 (49)
Radiology upon admission to hospital	
- Pneumonia	31 (43)
- TB	21 (29)
- Not Pneumonia and not TB	20 (28)
SARS CoV2 PCR screening results	
- Negative	59 (82)
- Positive	13 (18)
Treatment room	
- ICU	18 (25)
- Non-ICU	54 (75)
Total	72 (100)

FilmArray examination of 72 nasopharyngeal and oropharyngeal swab specimens revealed no atypical bacteria or a combination of viruses and bacteria. Three types of viruses were detected, as detailed in Table 2.

Table 2. FilmArray examination results

Characteristics	Frequency (%)
Virus	27 (38)
- Human rhinovirus / Enterovirus (HRV/E)	13 (48)
- Coronavirus (SARS-CoV2)	13 (48)
- Human metapneumovirus (HMP)	1 (4)
Atypical Bacteria	0 (0)
Double infection of Virus and Bacteria	0 (0)
Not detected	45 (62)
Total	72 (100)

DISCUSSION

The results of a study of 72 subjects with respiratory tract infections showed that the productive age group (15-64 years) had a higher frequency than the non-productive age group (>64 years). This is consistent with the results of the Indonesian national health survey in the 2018 Basic Health Research (Riskesdas), which showed 5.51% of the productive age group compared to 0.55% of the non-productive age group, out of 1,017,290 respondents. Meanwhile, similar results were also obtained by the 2023 Indonesian Health Survey (SKI), where of 877,531 survey respondents, 23.55% were productive age respondents and 14.61% were non-productive age respondents.^{6,13}

In this study, there was no significant difference in the number of men and women hospitalized for respiratory infections. The 2018 Basic Health Research (Riskesdas) and

the 2023 Indonesian Health Survey (SKI) also showed no significant difference in the ratio of men and women to ARI patients.^{6,13}

Radiological findings of pneumonia were found in 43% of study subjects, a higher rate than the 2018 Riskesdas and 2023 SKI, which showed only 3.91% and 10.79% of clinical pneumonia cases among all survey respondents. This may be due to different data collection methods. Riskesdas and SKI were based on community respondents using questionnaires, while this study was based on patient medical records in hospitals. Similar results are also reflected in a study in China, which found 28.5% of pneumonia cases detected in hospitalized respiratory tract infections.¹⁴ Meanwhile, 28% of non-TB and non-pneumonia cases in this study were not actually pneumonia. This finding is similar to other studies in China, where 19% and 31% of respiratory tract infection cases were not pneumonia.^{14,15}

The COVID-19 pandemic has taught us many lessons about the crucial role of laboratories as a pillar of early detection and laboratory-based surveillance. Confirmation of laboratory test results is essential for detecting emerging disease cases. Advanced molecular methods like FilmArray, which provide rapid detection results in a single test, have shown that pathogens previously not routinely detected due to capacity limitations can be identified more accurately and quickly.

This study detected three types of viruses that cause respiratory tract infections: human rhinovirus/enterovirus (48%), SARS-CoV2 (48%), and human metapneumonia virus (4%), as single detections without any other viruses or bacteria. These findings align with a 2017 study in China that found RSV to be the most prevalent virus in community-acquired pneumonia (CAP) patients, followed by human rhinovirus and influenza virus.¹⁶ Research in the UK also found that Rhinovirus is the most common after influenza virus.¹⁷ The use of other respiratory panel FilmArrays also found that the most common viruses were influenza followed by Rhinovirus,¹⁸ while a multicenter FilmArray study found the most prevalent were HRV/EV, RSV, adenovirus, and FluA.¹⁹

Thirteen positive SAR-CoV2 FilmArray results corresponded to thirteen detection results from real-time PCR screening of Covid-19 that detected SARS-CoV2 in the same samples, indicating that the FilmArray method has the same capability in terms of detection with a faster time. In a study using 30 clinical samples and 27 tests for the limit of detection test, the results were 100% concordant with the results of the examination using the real-time PCR kit developed by the examining laboratory.¹² Similar results were also obtained from another multicenter study in the United States with three different states which showed positive percent agreement of 98.4% and negative percent agreement of 98.9%.²⁰

The failure to detect influenza virus may be due to viral levels below the limit of detection (LOD) and the sampling period, which mostly occurred from May to June. Based on Indonesian Ministry of Health ILI and SARI surveillance data (2020–2024), influenza cases generally increase at the end and beginning of the year. The 45 undetected samples may also indicate respiratory infections outside the pathogen spectrum covered by the respiratory panel. This study recorded 31 pneumonia cases and 21 TB cases.

Various bacteria, viruses and even fungi can cause respiratory tract infections or pneumonia, such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Epstein-Barr virus*, *Pneumocystis jirovecii* and so forth.²² In the etiological surveillance of respiratory tract infections in China from 2009 to 2019, the above pathogens were also detected along with other pathogens that were within the detection capacity of the FilmArray method.¹⁴ Meanwhile, in another study that also used a respiratory panel FilmArray, 23.2% of negative results detected *Aspergillus* spp.,

Mycoplasma pneumoniae, *Mycobacterium tuberculosis*, *Enterobacter cloacae*, and *Candida tropicalis*.¹⁸

This study provides information that the pathogen causing pneumonia will be identified more quickly with the FilmArray method because of its ability to detect various atypical viruses and bacteria causing respiratory tract infections, both as single infections and as a combination of infections in a faster time.^{11,12} This will aid in patient management. A limitation of this study is that it did not analyze the results of other etiological tests for comparison.

CONCLUSION

This study demonstrated that a molecular-based method using the FilmArray respiratory panel can be used to quickly and accurately detect microorganisms in respiratory tract infections, thereby expediting patient diagnosis and treatment. This research is highly useful for early detection of pneumonia-causing pathogens and can be used to alert for coinfections that can worsen pneumonia.

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