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Identification of Community- and Hospital Pulmonary Bacterial Infection Using Culture and PCR Panel in COVID-19

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ABSTRACT

Co-infection or secondary infection is associated with a worse outcome in COVID-19. Information concerning the distribution of pathogenic microbes in COVID-19 has yet to be widely studied. This study aims to evaluate the distribution of bacterial infection in COVID-19, detected using conventional culture and molecular methods. This study was conducted in March-May 2021 in Dr. Hasan Sadikin General Hospital, with a study population of moderate, severe, and critical COVID-19 patients. Microorganisms were identified and analyzed from expectorant sputum or Endotracheal tube aspirates using conventional culture methods (VITEK 2 Compact) and multiplex PCR pneumonia panel (Biofire). Data was presented in a table and figures to describe the organism profile among the two methods. From the 450 COVID-19 patients, 59 subjects were included. The positivity rate of microbial identification reached 79.7% in both methods, dominated by Gram-negative bacteria for both community and hospital-acquired infections. The pathogens most frequently detected using conventional methods and multiplex PCR were *Acinetobacter baumanii* (15.3%; 23.7%) and *Klebsiella pneumoniae* (23.7%; 28.8%). The multiplex PCR method detected *Haemophilus influenzae* (15.3%) and respiratory viruses (3.4%), which conventional methods could not detect. Gram-negative bacteria were the most frequent pathogen in COVID-19 in both populations. The multiplex PCR method has the advantage due to its shorter examination time. The application of both methods helps determine antibiotic therapy for COVID-19. Both methods identified *Klebsiella pneumoniae* and *Acinetobacter baumanii* as the dominant bacteria in both populations. This study helps establish antibiotic management in COVID-19, thus preventing antibiotic resistance.

Keywords: Co-infection, COVID-19, Gram-negative bacteria, multiplex PCR pneumonia panel

INTRODUCTION

The World Health Organization (WHO) has declared Coronavirus Disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) infection as a global pandemic in January 2020.[1] In general, the clinical presentation of COVID-19 can be asymptomatic or symptomatic. The common symptoms of COVID-19 are fever, fatigue, and dry cough. In addition, other symptoms may appear, i.e., myalgia, rhinorrhea, headache, conjunctivitis, sore throat, diarrhea, anosmia, and skin rash. However, severe cases of COVID-19 may result in acute respiratory failure, kidney failure, and even death.[2]

Around 40% of COVID-19 cases have mild symptoms, the other 40% have moderate symptoms such as pneumonia, 15% have severe symptoms, and 5% have severe symptoms causing critical condition. Patients with minor symptoms were reported to have recovered within a week. In severe cases, patients could suffer from Acute Respiratory Distress Syndrome (ARDS), sepsis, septic shock, or multi-organ failure, including renal or acute heart failure, which can lead to death. People over the age of 65 (elderly), as well as those with comorbid conditions, such as hypertension, heart and lung disease, diabetes, or cancer, are at a higher risk of manifesting worse symptoms.[3,4]

Co-infection or secondary infection of pathogenic microbes in COVID-19 increases COVID-19 severity. However, research on pathogenic microbe infection in COVID-19 has yet to be studied.[5] COVID-19 can potentially increase antibiotic use, which can contribute to increased antibiotic resistance in the long run.[6] The Antibiotics Susceptibility Test (AST) using conventional culture technique is commonly used to assess antibiotic prescriptions. However, this approach has the disadvantage of taking a long time to complete and a limited variety of pathogens that can be cultured.[7] This restriction could have an impact on the increase of antibiotic prescriptions among COVID-19 patients.[8,9]

A molecular detection panel (Biofire FilmArray system), a rapid and fully automated multiplex PCR

classified as a Rapid Molecular Test (RMT), has been developed. One of the advantages of this examination is its ability to detect multiple pathogenic microbes in a single examination. This examination also provides information on antimicrobial gene resistance.[7,10] This study aimed to observe the distribution of pathogenic microbes found in the COVID-19 patient population using a molecular panel and conventional culture media.

METHODS

The subjects of this study were patients diagnosed with COVID-19 pneumonia, based on Indonesia's national guidelines for COVID-19 diagnosis management.[4] The following criteria were used to determine the inclusion in this study: Adult patients, age of 18 and above; Confirmed COVID-19 patient ≤ 10 days since admission to the hospital, based on a nasopharyngeal swab examination using real-time PCR; Patients with moderate, severe, or critical COVID-19 symptoms; Patients with clinical suspicion of co-infection or secondary infection as determined by the clinician. The following patients were excluded from this study: Those who had received treatment in intensive care before being confirmed with a COVID-19 diagnosis; Those who had repeated hospital admission (more than twice) within the previous three months. Community infections were groups of patients with treatment durations of less than 48 hours at the time of specimen collection. In contrast, hospital infections were defined as groups of patients with treatment durations of more than 48 hours at the time of specimen collection.[11]

Patients who met the study criteria were informed about their participation and asked for their consent to participate (informed consent). If the patient could not express their choice, the questions were asked to a relative or authorized guardian. The number of subjects in this study was determined by the observation period between March and May 2021 (3 months) or by achieving a minimum of 30 research subjects. We selected and categorized the patient characteristics into age (based on 60 years), disease severity according to COVID-19 guidelines, onset of infection, and specimen type.[4] The conventional culture methods for organism identification were performed using Vitek2 Compact (Biomerieux, France); meanwhile, rapid multiplex PCR was performed using Biofire (Biomeriuex, France) with a pneumonia panel kit. The procedure for performing organism identification was done according to the Clinical Laboratory Standard Institute (CLSI) guidelines and manufacturer recommendations, including quality assurance of each method. This study used ethical clearance issued by the Research Ethics Committee Universitas Padjadjaran Bandung, No. 387/UN6.KEP/EC/2021. Study variables, i.e., age, gender, disease severity, onset of infection, and specimen type, were presented in frequencies and percentages. Organism identification based on two methods was presented in a graph and drawn using STATA 12.0 (Stata, Texas, USA).

RESULTS AND DISCUSSIONS

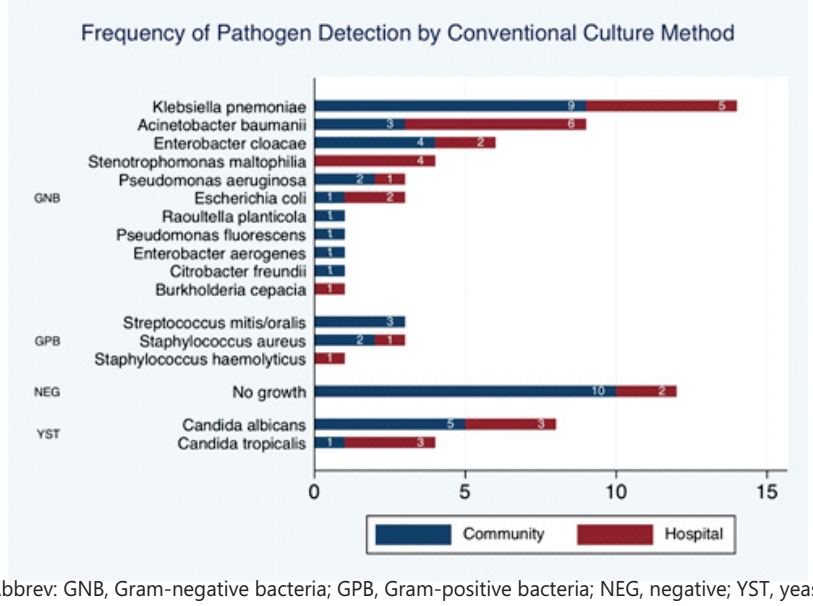
Four hundred fifty moderate, severe, and critical COVID-19 patients were at Dr. Hasan Sadikin General Hospital from March to May 2020. Fivety-nine subjects met the research criteria; the characteristics are shown in Table 1.

Table 1. Patient characteristics data

Variable	Total (n=59)	
	n	(%)
Age (years)		
< 60	26	44.1
>= 60	33	55.9
Gender		
Male	30	50.8
Female	29	49.2
Disease severity		
Moderate	22	37.3
Severe	28	47.5
Critical	9	15.2
Onset of infection		
Community onset (<= 48h)	36	61.0
Hospital onset (> 48h)	23	39.0
Specimen type		
Expectorate sputum	52	88.1
Endotracheal aspirate	7	11.9

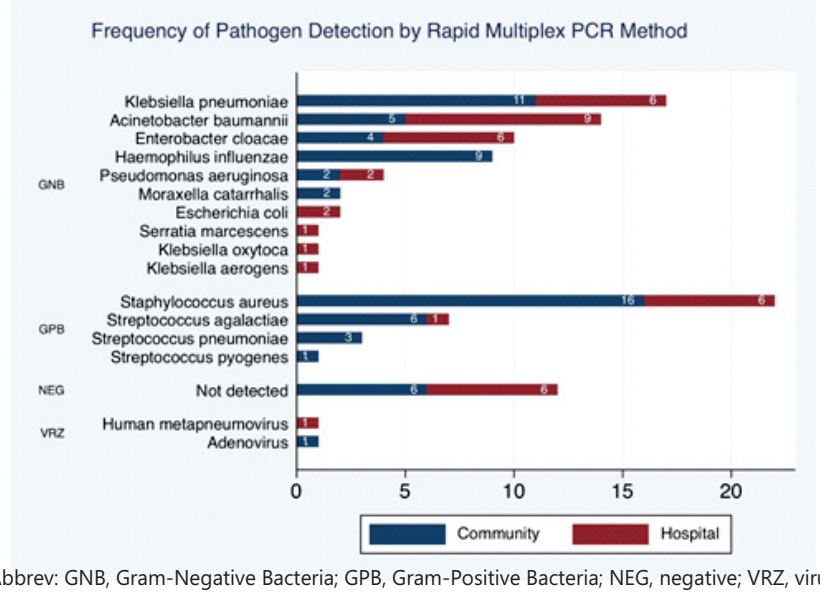
Abbrev: h, hours

As shown in Table 1, 55.9% of the subjects were 60 or older, with an even gender distribution (30 male subjects and 29 female subjects). Most of the samples collected in this study came from patients' sputum (88.1%). The conventional culture method identified microorganisms in 47 isolates (79.7%), including 27/36 (75.0%) in the community-acquired infection group and 20/23 (87.0%) in the hospital-acquired infection group. Gram-negative bacteria (*Klebsiella pneumoniae* (23.7%), *Acinetobacter baumanii* (15.3%), and *Enterobacter cloacae* (15.3%)) were the most commonly identified pathogens in the culture method (10.1%) (Figure 1).



Abbrev: GNB, Gram-negative bacteria; GPB, Gram-positive bacteria; NEG, negative; YST, yeast

Figure 1. Pathogen identification with conventional culture methods



Abbrev: GNB, Gram-Negative Bacteria; GPB, Gram-Positive Bacteria; NEG, negative; VRZ, virus

Figure 2. Pathogen identification through rapid multiplex PCR

The rapid multiplex PCR examination identified microorganisms on 47 samples (79.7%), including 30/36 (83.3%) in the community-acquired Infection group and 17/23 (73.9%) in the hospital-acquired infection group. The most common pathogens identified through the rapid multiplex PCR method were mainly Gram-negative bacteria, such as *Klebsiella pneumoniae* (28.8%), *Acinetobacter baumannii* (23.7%), *Enterobacter cloacae* (16.9%), *Haemophilus influenzae* (15.3%), as well as Gram-positive bacteria, i.e., *Staphylococcus aureus* (37.3%) and *Streptococcus agalactiae* (11.9%) (Figure 2).

The differences in the identification of the two examination methods are shown in Table 2. Fungal infections such as *Candida albicans* (13.6%) and *Candida tropicalis* (6.8%) can only be detected through culture, while the virus can only be detected through the rapid multiplex PCR method. Bacteria that are difficult to grow in culture, such as *Haemophilus influenzae* (15.3%), can also be detected using the rapid multiplex PCR method.

This study evaluated the distribution of bacteria in COVID-19 patients using two methods: culture and rapid multiplex PCR. Both approaches have a high detection rate of 79.7%. Table 3 compares the

Table 2. Identification of differences between conventional methods and rapid multiplex PCR method

Microorganisms Detected Only Through Conventional Methods	Total n=59 n (%)	Microorganisms Were Detected Only Through the Rapid Multiplex PCR Method	Total n=59 n (%)
Candida albicans[a]	8 (13.6)	*Haemophilus influenza*	9 (15.3)
Candida tropicalis	4 (6.8)	*Streptococcus agalactiae*	7 (11.9)
Stenotrophomonas maltophilia[a]	4 (6.8)	*Streptococcus pneumoniae*	3 (5.1)
Streptococcus mitis/oralis[a]	3 (5.1)	*Moraxella catarrhalis*[b]	2 (3.4)
Staphylococcus haemolyticus[a]	1 (1.7)	*Adenovirus*[c]	1 (1.7)
Burkholderia cephacia[a]	1 (1.7)	*Human metapneumovirus*[c]	1 (1.7)
Citrobacter freundii[a]	1 (1.7)	*Serratia marcescens*	1 (1.7)
Enterobacter aerogenes[a]	1 (1.7)	*Klebsiella aerogens*	1 (1.7)
Pseudomonas fluorescens[a]	1 (1.7)	*Klebsiella oxytoca*	1 (1.7)
Raoultella planticola[a]	1 (1.7)	*Streptococcus pyogenes*	1 (1.7)

a Microorganisms not included in the Biofire FilmArray Pneumonia Plus Panel
b Fastidious bacterium that cannot be cultivated using routine media
c Virus that cannot be cultivated using routine media

Table 3. Detection rates comparison of culture and rapid multiplex PCR in previous research

Year	Country	Author	Sample Type	Study Population	Culture Detection Level	Multiplex PCR panel Detection Level
2020	South Korea	Yoo IY[12]	Sputum, ETT Aspirate	Patients of LRTI	65.7%	73.7%
2020	South Africa	Mitton B[13]	BAL, ETT Aspirate	Patients of LRTI with Respiratory failure	71.2%	71.2%
2021	Italy	Foschi C[14]	BAL, Bronchial Aspirate	Patients of COVID-19 in critical condition admitted to ICU	34.3%	40.0%
2021	Indonesia	This study	Sputum, ETT Aspirate	Patients of COVID-19 with moderate, severe, and critical conditions	79.7%	79.7%

Abbrev: LRTI, Lower Respiratory Tract Infections; ETT, Endotracheal Tube; BAL, Bronchoalveolar Lavage; ICU, Intensive Care Unit

detection rates for culture and rapid multiplex PCR with previous studies.

The rapid multiplex PCR method has several advantages, including a shorter examination time (2-3 hours), the ability to detect fastidious bacteria (*Haemophilus influenzae*), and the ability to detect resistance genes in bacteria. Some species, however, are not detectable using the rapid multiplex PCR method. As a result, additional evaluation using the culture method is still required. In this and previous studies, the rapid multiplex PCR method has the same or may have a higher detection rate than culture. This finding is because the rapid multiplex PCR method detects nucleic acids from bacteria in the sample, regardless of whether the bacteria are disease pathogens (true pathogens) or just colonization.[12,13]

The distribution pattern of microorganisms obtained in this study is similar to that of Garcia-Vidal *et al.*[15] They discovered that the most common causes of community co-infection were *Streptococcus pneumoniae* and *Staphylococcus aureus.* At the same time, the superinfection bacteria in hospitals were *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella spp.*, and *Staphylococcus aureus.*[15] The findings also show that Gram-negative bacteria dominated the distribution of bacteria in the Hospital Infection group, as obtained through rapid multiplex PCR and culture. *Acinetobacter baumanii* (25.6% in culture and 39.1% in rapid multiplex PCR) and *Klebsiella spp.* (21.7% in culture and 34.7% in rapid multiplex PCR) were the most common Gram-negative bacteria found in this study.

Enterobacter cloacae (26.1%) and *Staphylococcus aureus* (26.1%) were detected using the rapid multiplex PCR. However, due to its broad antibiotic resistance, the rapid multiplex PCR method could not detect *Stenotrophomonas maltophilia*, a nosocomial pathogen that is difficult to treat. Bacteria is also one of the causes of high mortality in patients.[16]

Gram-negative bacteria, specifically *Klebsiella pneumoniae* (25.0 %), *Enterobacter cloacae* (11.1%), and *Acinetobacter baumanii* (8.3%), dominated the distribution of bacteria in the hospital-acquired infection group in the culture method. On the other hand, the rapid multiplex PCR method detects the presence of *Klebsiella pneumoniae* (30.6%), *Enterobacter cloacae* (11.1%), *Acinetobacter baumanii* (13.9%), and *Haemophilus influenzae* (25.0%), as well as Gram-positive bacteria such as *Staphylococcus aureus* (44.4%) and *Streptococcus agalactiae* (16.7%) in the hospital-acquired infection group. The distribution of bacteria in this group indicates that pathogens in the hospital-acquired infections group are beginning to spread in the community. This result is likely due to the increased use of antibiotics as a response to the pandemic, especially at its early stage. In a meta-analysis study, Langford *et al.* discovered that 62.4% of COVID-19 patients used antibiotics.8 The study by Vaughn *et al.* also discovered that the use of antibiotics for COVID-19 patients reached 56.6% for empiric therapy in 38 hospitals in the United States.[9] This highlights the importance of reconsidering antibiotic use in COVID-19 patients.

It is challenging to determine empirical therapy in COVID-19 patients. COVID-19 patients have developed rapid symptoms; from the time of initial admission with minimal complaints, they can experience a worsening of the disease, such as respiratory failure, in a short time. The traditional method of bacterial identification is quite time-consuming (1-3 days). Meanwhile, the rapid multiplex PCR method has the advantage of identifying pathogens quickly, assisting clinicians in determining antibiotic therapy.

CONCLUSIONS AND SUGGESTIONS

The bacteria found in COVID-19 at Dr. Hasan Sadikin General Hospital in Bandung were mostly Gram-negative. The detection rate of the syndromic rapid multiplex PCR method was the same as that of the culture method (79.7%). Meanwhile, the rapid multiplex PCR method had a shorter examination time (2-3 hours), which helps clinicians determine the best antibiotic therapy.

REFERENCES

1. Lai C-C, Wang C-Y, Wang Y-H, Hsueh S-C, Ko W-C, Hsueh P-RJIjoaa. Global epidemiology of Coronavirus Disease 2019 (COVID-19): Disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status. Int J Antimicrob Agents, 2020; 55(4): 105946.
2. Lechien JR, Chiesa?Estomba CM, Place S, Van Laethem Y, Cabaraux P, *et al.* Clinical and epidemiological characteristics of 1420 European patients with mild?to?moderate Coronavirus disease 2019. J Intern Med, 2020; 288(3): 335-44.
3. Lansbury L, Lim B, Baskaran V, Lim WSJJoI. Co-infections in people with COVID-19: A systematic review and meta-analysis. J Infect, 2020; 81(2): 266-75.
4. Erlina Burhan ADS, Sally A Nasution, Eka Ginanjar, Ceva Wicaksono Pitoyo AS, Isman Firdaus, dkk. Protokol tatalaksana COVID-19. PDPI, PERKI, PAPDI, PERDATIN, IDAI, 2020; 1-49.
5. Hughes S, Troise O, Donaldson H, Mughal N, Moore LSJCM, Infection. Bacterial and fungal co-infection among hospitalized patients with COVID-19: A retrospective cohort study in a UK secondary-care setting. Clin Microbiol Infect, 2020; 26(10): 1395-9.
6. Rawson TM, Moore LS, Zhu N, Ranganathan N, Skolimowska K, *et al.* Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis, 2020; 71(9): 2459-68.
7. Monard C, Pehlivan J, Auger G, Alviset S, Dinh AT, *et al.* Multicenter evaluation of a syndromic rapid multiplex PCR test for early adaptation of antimicrobial therapy in adult patients with pneumonia. Crit Care, 2020; 24(1): 1-11.
8. Langford BJ, So M, Raybardhan S, Leung V, Soucy J-PR, *et al.* Antibiotic prescribing in patients with COVID-19: rapid review and meta-analysis. Clinical Microbiology and Infection. 2021; 27(4): 520-531.
9. Vaughn VM, Gandhi TN, Petty LA, Patel PK, Prescott HC, *et al.* Empiric antibacterial therapy and community-onset bacterial co-infection in patients hospitalized with coronavirus disease 2019 (COVID-19): A multi-hospital cohort study. Clinical Infectious Diseases, 2021; 72(10): e533-e41.
10. Lee SH, Ruan S-Y, Pan S-C, Lee T-F, Chien J-Y, *et al.* Performance of a multiplex PCR pneumonia panel for the identification of respiratory pathogens and the main determinants of resistance from the lower respiratory tract specimens of adult patients in intensive care units. J Microbiol Immunol Infect, 2019; 52(6): 920-8.
11. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, *et al.* Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clinical Infectious Diseases, 2016; 63(5): e61-e111.

12. Yoo IY, Huh K, Shim HJ, Yun SA, Chung YN, et al. Evaluation of the BioFire FilmArray pneumonia panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens. International Journal of Infectious Diseases, 2020; 95: 326-31.
13. Mitton B, Rule R, Said M. Laboratory evaluation of the BioFire FilmArray pneumonia plus panel compared to conventional methods for the identification of bacteria in lower respiratory tract specimens: A prospective cross-sectional study from South Africa. Diagnostic Microbiology and Infectious Disease, 2021; 99(2): 115236.
14. Foschi C, Zignoli A, Gaibani P, Vocale C, Rossini G, *et al*. Respiratory bacterial co-infections in intensive care unit-hospitalized COVID-19 patients: Conventional culture vs. BioFire FilmArray pneumonia plus panel. Journal of Microbiological Methods, 2021; 106259.
15. Garcia-Vidal C, Sanjuan G, Moreno-García E, Puerta-Alcalde P, Garcia-Pouton N, et al. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: A retrospective cohort study. Clinical Microbiology and Infection, 2021; 27(1): 83-8.
16. Patterson SB, Mende K, Li P, Lu D, Carson ML, *et al*. *Stenotrophomonas maltophilia* infections: Clinical characteristics in a military trauma population. Diagnostic Microbiology and Infectious Disease, 2020; 96(2): 114953.