Tropical Biomedicine 42(1): 21-26 (2025)
https://doi.org/10.47665/tb.42.1.004

RESEARCH ARTICLE

Use of multiplex molecular respiratory panel in COVID-19 patients with suspected co-infections: Insights and considerations in results interpretation

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ARTICLE HISTORY

Received: 7 August 2024
Revised: 15 November 2024
Accepted: 19 November 2024
Published: 24 February 2025

ABSTRACT

As critically ill COVID-19 patients are prone to infections by other respiratory pathogens, this study aimed to investigate the detection of respiratory co-pathogens in such patients, by employing a multiplex respiratory molecular panel. Conducted at Universiti Sains Malaysia Specialist Hospital (HPUSM) from November 1, 2021, to November 1, 2022, this retrospective cross-sectional study analysed adults admitted with confirmed COVID-19. Oropharyngeal/ nasopharyngeal swabs collected upon admission were tested using the Fast Track Diagnostic (FTD®) Respiratory Pathogens 33 multiplex kit to detect other respiratory pathogens. Within 48 hours of admission, the presence and type of organisms were determined to assess community co-infections. Among 48 critically ill COVID-19 patients (26 male; mean age, 62.2 years), clinically significant organisms were found in 32 patients (67%). Bacterial co-infections or co-colonization were detected in 91% (29/32) of these patients, with *Klebsiella pneumoniae* (40%) and *Staphylococcus aureus* (23%) were the most common, followed by *Streptococcus pneumoniae* (11%), *Moraxella catarrhalis* (4%), and *Haemophilus influenzae* (2%). Fungal (6%) and viral (2%) co-infections were less frequent. The use of syndromic respiratory panel in critically ill COVID-19 patients offers the advantage of being highly sensitive, rapid, and able to detect multiple pathogens. However, detection of multiple respiratory pathogens needs to be interpreted with caution, taking into consideration patient's clinical and radiological findings, as well as quantitative molecular data.

Keywords: SARS-CoV-2; COVID-19; co-detection; co-infection; respiratory pathogen.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a global pandemic contributed by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-sense, single-stranded RNA virus with a genome size of around 29 – 30 kb. The high infectivity rate of SARS-CoV-2 facilitates rapid and widespread transmission worldwide. As of now, the virus has infected over 5 million people in Malaysia, resulting in more than 37,000 deaths across the region (Ministry of Health Malaysia, 2024).

COVID-19 co-infection is denoted when a patient is infected with SARS-CoV-2 and other bacterial or viral respiratory pathogens at the same time. In regard to the ongoing emergence of novel SARS-CoV-2 variants, it is important to have a clear understanding of the epidemiology and clinical implications of COVID-19 co-infections. Despite the mild and asymptomatic cases of the current reported COVID-19 worldwide, the severity of the disease may still be exacerbated by the presence of other co-infecting organisms (Alhumaid *et al*., 2022). The global incidence of bacterial and viral co-infections with COVID-19 was reported to be around 3.02 – 20.2% and 5.41 – 6.61%, respectively (Alhumaid *et al*., 2022; Fan *et al*., 2023).

However, the proportion and types of pathogens vary among studies. Common bacteria associated with COVID-19 co-infections are *Pseudomonas aeruginosa*, *Klebsiella* spp., and *S. aureus* (Fan *et al*., 2023; Patton *et al*., 2023). Meanwhile, viral co-infections are predominant among influenza viruses, respiratory syncytial virus (RSV), and adenoviruses (Swets *et al*., 2022). Both bacterial and viral co-infections had been reported to have a profound impact on the increased likelihood of in-hospital mortality, intensive care unit admission, and the requirement for invasive mechanical ventilation, compared with SARS-CoV-2 single infection (Patton *et al*., 2023; Swets *et al*., 2022). A study from Thailand has reported 45% of patients had co-infection with 18.8% had bacterial co-

infection (Pongpirul *et al*., 2020). While in Malaysia, Chong *et al*. (2021) reported that only 8.9% of 56 COVID-19 positive samples had documented respiratory virus co-infection mainly from rhinovirus and enterovirus (Chong *et al*., 2021).

A study to detect respiratory co-pathogens in the local settings will aid to understand better the correlation between COVID-19 and respiratory co-infection. Rapid diagnostic tests for the detection of respiratory co-pathogens among critically ill COVID-19 patients on admission can play a pivotal role in managing empirical therapy and antimicrobial stewardship. Hence, this study was conducted to evaluate the presence of respiratory co-pathogens either viruses, bacteria and/ or fungal, among critically ill COVID-19 patients.

MATERIALS AND METHODS

Study design and ethical approval

This retrospective, cross-sectional, single-center study was performed from 1st November 2021 to 1st November 2022 at Universiti Sains Malaysia Specialist Hospital (HPUSM), Malaysia. The study protocol was reviewed and approved by Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/ COVID19-44). The Declaration of Helsinki and Good Clinical Practice were followed during the study's execution.

Clinical data and samples collection

Participants were patients who were diagnosed as critically ill (CAT 5) COVID-19 patients who were suspected of having co-infection. From this study, CAT 5 is defined as critically ill patients with confirmed COVID-19 who requires mechanical ventilation, shock requiring inotrope, or multi-organ failure (Ministry of Health Malaysia, 2023). The inclusion criteria for participants were age 18 years and above, laboratory diagnosis confirmed with SARS-CoV-2 infection based on positive SARS-CoV-2 PCR or GeneXpert® from nasopharyngeal/ oropharyngeal swabs, clinical severity of CAT 5 within 48 hours of admission, and diagnosed between 1st November 2021 until 1st November 2022. The exclusion criteria were patients with laboratory diagnosis of SARS-CoV-2 based on rapid test antigen testing and history of past COVID-19 co-infection of more than 3 months (repeated infection).

Their combined oropharyngeal/ nasopharyngeal swabs collected on admission were sent to the Laboratory of Medical Microbiology and Parasitology, School of Medical Sciences for molecular testing. The demographic and clinical data of patients; comorbid conditions; laboratory results, vaccination status; in-hospital management and outcomes were traced from medical record unit and hospital laboratory information system.

Samples preparation and processing

The Nextractor®NX-48N (Genolution Inc., Seoul, Korea) instrument and its extraction kit was used to extract RNA by following the manufacturer's instructions. An elution volume of 60 ml of RNA were obtained from each samples during per extraction round and was stored in 1.5 ml microcentrifuge tubes at –80°C. Detection of respiratory pathogens were performed within a week after RNA extraction.

Detection of respiratory pathogens

The detection of respiratory pathogens was performed on the Bio-Rad CFX96™ (Bio-Rad Laboratories, Inc., California, USA), utilising the Fast Track Diagnostic (FTD®) Respiratory Pathogens 33 multiplex kit (Siemens Healthcare SA, Esch-sur-Alzette, Luxembourg), which is a commercial reverse transcriptase real-time polymerase chain reaction (RT-qPCR), one-step RT-qPCR method. A 10 ml of extracted RNA was added into the 15 ml of master mix containing 12.5 ml of buffer, 1.5 ml of the primer-probe mix, and 1 ml of the enzyme. The FTD®-33 assay protocol was as follows: hold step (50°C for 15 mins), hold step (94°C for 1 min), cycling step (94°C for 8 secs followed by 60°C for 1 min) for 40 cycles.

This FTD®-33 amplification kit detects simultaneously 21 respiratory viruses: influenza A – C viruses (FluA, FluB and FluC), influenza A swine virus (H1N1), human rhinovirus (RV), human coronaviruses (NL63, 229E, OC43, HKU1), human parainfluenza 1–4, human metapneumoviruses A/B (HMPVA/B), human bocavirus (HBoV), human respiratory syncytial viruses A/B (HRSVA/B), human adenovirus (HAdV), enterovirus (EV), and human parechovirus (HPeV), and 12 respiratory bacteria: *P. jirovecii, Chlamydophila pneumoniae, S. pneumoniae, Haemophilus influenzae B*, *S. aureus, Moraxella catarrhalis, Bordetella* spp.* except *Bordetella parapertussis*, *K. pneumoniae*, *Legionella pneumophila/ Legionella longbeachae*, *Salmonella* spp., and *Haemophilus influenzae*. The internal control (IC) of the kit is equine arteritis virus (EAV).

A sample was considered positive for a pathogen at any sigmoidal curve within a cycle threshold (C_T) value of < 40. An IC evaluated both nucleic acid extraction and PCR inhibition. The IC was extracted with the specimens and utilised with each PCR run along with positive and negative controls provided by the manufacturers.

Statistical analysis

All continuous variables are expressed as mean ± standard deviation (SD) and categorical variables are expressed as numbers (percentages). The Chi-square test or Fisher's exact test was utilised to assess the association between the two categorical variables. A p-value < 0.05 was considered as significant according to one- or two-sided tests. All statistical analysis was performed using IBM SPSS® Statistics version 26.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Comparison of clinical characteristic and laboratory data between COVID-19 single detection and co-detections group

This study has recruited 48 critically ill COVID-19 patients classified as CAT 5 according to the inclusion and exclusion criteria. Demographic and clinical data of COVID-19 (CAT 5) patients were collected from HPUSM after obtaining ethical approval from JEPeM-USM. According to Table 1, 16 COVID-19 (CAT 5) patients were aged < 60 years old and 32 patients were aged > 60 years old, with an average mean age of 62.2 at the time of diagnosis.

Gender distribution was even, with 54% were males (n = 26) and 46% were females (n = 22), resulting in an almost equivalent male-to-female ratio of 1.18:1. The patient population was racially homogenous, with 98% (n = 47) being of Malay ethnicity, and only one of them were Indonesian (other ethnicity).

Eighty-four percent (n = 29/32) of co-pathogens detection patients had at least one medical comorbidity upon admission, complicating their disease. 71% of COVID-19 (CAT 5) patients were having hypertension (n = 23), followed by 68% of patients were having diabetes mellitus (n = 22), and 38% of patients were having chronic kidney disease (n = 12). However, there was no significant differences between single pathogen detection and co-pathogens detection groups in terms of comorbidities.

Regarding vaccination status upon admission, majority of COVID-19 (CAT 5) patients (65%, n = 31) were unvaccinated, defined as having never received any COVID-19 vaccine. Twenty-seven percent (n = 13) had completed the vaccination twice, while only 8% had completed it once (n = 4). Among co-detections group, 69% (n = 22) were unvaccinated, but the was no significant association between vaccination status and co-pathogens detection group (p = 0.066).

Despite the notable prevalence of bacterial respiratory co-pathogens detection as detected by the FTD®-33 PCR assay, the majority of patients did not exhibit positive blood cultures. Specifically, only 6% (n = 3) of blood cultures were positive,

Table 1. Socio-demographic and clinical parameters of the study population

Variables	Total patients *n* = 48 (%)	Co-pathogen detection *n* = 32 (%)	No co-pathogen detection *n* =16 (%)	*p* value
Demographic data				
Age (mean ± SD)	62.2	60.3 ± 13.8	65.8 ± 12.6	0.189
18 – 39 years old	4 (8)	3	1	0.303
40 – 59 years old	12 (25)	10	2	
> 60 years old	32 (67)	19	13	
Gender				
Male	26 (54)	17 (53)	9 (56)	0.838
Female	22 (46)	15 (47)	7 (44)	
Ethnicity				
Malay	47 (98)	31 (97)	16 (100)	
Chinese	0	0	0	
Indian	0	0	0	
Others	1 (2)	1 (3)	0	
Comorbidities				
Diabetes mellitus	34 (71)	22 (69)	12 (75)	0.653
Hypertension	38 (79)	23 (71)	15 (94)	0.079
Ischemic heart disease	13 (27)	5 (16)	8 (50)	**0.012**
Chronic kidney disease	21 (44)	12 (38)	9 (56)	0.338
Bronchial asthma	3 (6)	2 (6)	1 (6)	1.000
COPD	1 (2)	1 (3)	0	0.475
Others	17 (35)	11	6	
No comorbidity	3 (6)	3	0	
Vaccination status				
Twice	13 (8)	6 (19)	7 (44)	0.066
Once	4 (27)	4 (12)	0 (0)	1.563
Unvaccinated	31 (65)	22 (69)	9 (28)	0.729
Clinical and laboratory data on hospital admission				
Total white cell means (× 10^9/L)	13.24 ± 7.3	13.24 ± 7.8	13.24 ± 6.6	1.000
Leucocytosis (> 11× 10^9/L)	28 (58)	17 (53)	9 (56)	0.838
Leukopenia (< 4×10^9/L)	2 (4)	2 (6)	1 (6)	1.000
Absolute Lymphocyte Count (ALC) (× 10^9/L)	1.75 ± 4.32	1.89 ± 5.17	1.42 ± 1.49	0.764
ALC < 1000	27(56)	20 (63)	7 (44)	0.216
Mean Absolute Neutrophil Count (ANC) × 10^9/L	11.18 ± 6.52	10.77 ± 6.71	12.06 ± 6.25	0.520
Mean Neutrophil / Lymphocyte ratio (NLR)	16.48 ± 17.23	17.07 ± 18.67	15.20 ± 14.17	0.726
NLR > 6	34 (71)	22 (69)	12 (75)	0.263
NLR > 18	15 (31)	11 (34)	4 (25)	0.314
Mean Hemoglobin (g/dl)	11.81 ± 2.41	12.17 ± 2.66	11.08 ± 1.91	0.151
Hb < 10g/dl	12 (25)	8 (25)	4 (25)	1.000
Mean Platelet Count	264 ± 137	263 ± 118	264 ± 173.61	0.987
CRP level (mg/l)				
< 10mg/l	3 (6)	2 (6)	1 (6)	0.262
> 50mg/l	32 (67)	21 (65)	11 (69)	0.297
Mean lactate dehydrogenase (LDH) (IU)	1528 ± 3576	1038 ± 627	2574 ± 6279	0.173
Mean albumin (g/dl)	34.8 ± 6.0	34.9 ± 5.1	34.3 ± 7.6	0.746
Hypoalbuminemia (< 35g/dl)	27 (56)	19 (41)	8 (50)	0.537
Mean aspartate aminotransferase (AST) (IU)	161.35 ± 662.21	76.66 ± 159.69	330.75 ± 1129.36	0.214
Mean alanine aminotransferase (ALT) (IU)	137.98 ± 636.65	53.81 ± 71.35	306.31 ± 1101.89	0.198
High AST (> 35IU)	30 (63)	21 (66)	9 (56)	0.527
High ALT (> 35IU)	18 (38)	13 (41)	5 (31)	0.527
Mean alkaline phosphatase (ALP) (IU)	120.56 ± 65.04	113.66 ± 53.42	134.38 ± 83.97	0.303
Mean Total Bilirubin (TB)	16.54 ± 16.57	14.72 ± 13.55	20.19 ± 21.43	0.285
Antibiotic receipt on admission	31 (65)	20 (63)	11 (69)	0.670
ETT culture growth	1/12 (8)	1 (3)	0	
Blood culture with growth	3 (6)	1 (3)	2 (12)	
Blood culture no growth	45 (94)	30 (94)	14 (88)	

identifying methicillin-sensitive *S. aureus* (MSSA), *Candida albicans*, and one possibly contaminant methicillin-resistant coagulase-negative *Staphylococcus* (MRCONS). *C. albicans* was detected in blood cultures, which was interpret as indicative of invasive candidiasis, rather than mere colonization, since *C. albicans* is typically a colonizer in the upper respiratory tract. Its presence in a blood culture suggests that the pathogen may have invaded a sterile sites, as opposed to being merely part of the microbiota. This interpretation is consistent with findings from Ramli *et al*. (2023), who reported *C. albicans* as a common pathogen in blood cultures of COVID-19 patients, while it was not detected in respiratory samples (Ramli *et al*., 2023). Regarding the other pathogens identified, MSSA is known pathogen and its detection in blood cultures suggests a possible bacterial bloodstream infection. In contrast, MRCONS was identified in one of the blood cultures but is often considered a contaminant in blood cultures, particularly when identified in low quantities or from a single sample, as in this case.

Additionally, the endotracheal tube (ETT) culture showed a mere 2% (n = 1) positivity, with *K. pneumoniae* being the identified pathogen, coinciding with its detection by the FTD®-33 PCR assay. This low positivity rate in the ETT cultures is consistent with several factors. First, COVID-19 co-infections remains relatively uncommon, particularly in the Malaysian hospital setting. According to a study by Ramli *et al*. (2023), the prevalence of COVID-19 co-infection is estimated to be 2.3%, suggesting that the majority of patients with COVID-19 do not have secondary bacterial infections (Ramli *et al*., 2023). As a result, most culture results in this study were negative. Moreover, molecular diagnostic test, the FTD®-33 PCR assay used in this study, are often more sensitive than traditional culture methods and may have been more effective in detecting co-infections, particularly in respiratory specimens. This helps explain the discrepancy between the low positivity rate of cultures and the potential presence of co-infections, which may not always be captured by culture techniques. Finally, since COVID-19 primarily affects the respiratory system, bacterial co-pathogens are often localized to the respiratory tract, making it less likely for these pathogens to invade the bloodstream. As such, blood cultures are typically less likely to yield positive results, particularly when the bacterial infections does not disseminate into the bloodstream.

Empirical antibiotics were administered to 65% (n = 31) of COVID-19 (CAT 5) patients within 48 hours of admission. Among these, 65% (n = 20) had co-pathogens detection, while 69% (n = 11) of patients without co-pathogens detection also received empirical antibiotics within the specified timeframe.

Regarding the clinical outcomes within the initial 48 hours of admission, a significant portion of patients required mechanical ventilation (67%), with 73% necessitating inotropic support. Upon discharge, only 25% were alive. Although there were no significant differences observed between single pathogen detection and co-pathogens detection groups regarding the need for mechanical ventilation, inotropic requirement, and death on discharge, it is noteworthy that more patients in the co-pathogens detection group required inotropes and mechanical ventilation, and the number of deaths were higher.

Co-detection of SARS-CoV-2 with other respiratory pathogens

A significant proportion of respiratory co-pathogen were detected within 48 hours of admission in this study. According to Table 2, among the 48 samples examined using the FTD®-33 PCR assay, 32 out of 48 samples (67%) tested positive for at least one respiratory pathogen, including viruses, bacteria, or fungi. Bacterial co-pathogens were predominant, constituting 91% (29/32) of the co-detection cases, followed by fungal at 6% (2/32), and virus at 2% (1/32).

Notably, six patients exhibited dual bacterial co-pathogens were detected in their samples, while one patient displayed triple bacteria co-pathogens detected, and another had quadruple bacteria co-pathogens detected. Among bacterial detection (Table 3), *K. pneumoniae* emerged as the most prevalent etiologic agent, accounting for 40% (19/48) of cases, constituting 60% of the co-pathogens detected (19/32). *S. aureus* detection ranked second at 23% (11/48), followed by *S. pneumoniae* at 11% (7/48), *M. catarrhalis* at 4% (2/48) and *H. influenzae* at 2% (1/48).

In terms of viral pathogen (Table 3), human RSV was detected in only one patient, while fungal pathogen (Table 3) revealed two patients testing positive for *P. jirovecii*. Regarding the baseline clinical characteristics, demographics, laboratory data, vaccination status, and clinical outcomes of the patients, no statistically significant differences were observed between single detection and co-detection groups ($p > 0.05$ for all variables), except for the presence of ischemic heart disease (IHD), which showed a statistically significant association ($p = 0.012$). This finding suggests that IHD is significantly associated with a higher likelihood of experiencing co-infections in COVID-19 patients, compared to having just a single infection. This is in line with previous research indicating that patients with underlying cardiovascular conditions, such as coronary heart disease (CAD), are at greater risk for severe outcomes and complications of COVID-19 (Hajikhani *et al*., 2023). A systematic review and meta-analysis by Hajikhani *et al*. (2023) found that CAD is prevalent in a significant proportion of COVID-19 patients and that the simultaneous presence of both CAD and COVID-19 increases the risk of complications. Specifically, the elderly patients and men with both conditions were found to experience a higher incidence of complications, supporting the idea that underlying cardiovascular conditions like IHD may contribute to greater vulnerability to co-infections (Hajikhani *et al*., 2023).

Table 2. Detection of SARS-CoV-2 co-detection with other pathogens

Detection of pathogens	Types of pathogen	Number (n)
Single bacterial pathogen	*K. pneumoniae*	13
	S. aureus	5
	S. pneumoniae	2
	M. catarrhalis	1
	H. influenzae	1
Single fungal pathogen	*P. jirovecii*	2
Bacterial co-pathogens	*K. pneumoniae* + *S. aureus*	3
	K. pneumoniae + *S. pneumoniae*	1
	S. aureus + *S. pneumoniae*	1
	M. catarrhalis + *S. pneumoniae*	1
	K. pneumoniae + *S. aureus* + *S. pneumoniae*	1
Viral and bacterial co-pathogens	Human RSV + *K. pneumoniae* + *S. aureus* + *S. pneumoniae*	1

Table 3. PCR mean CT values of pathogens detected

Pathogens	Number of patients	Percentage of detection (%)	PCR mean CT values
K. pneumoniae	19	40	36.43
S. aureus	11	23	35.86
S. pneumoniae	7	15	30.83
M. catarrhalis	2	4	34.14
P. jirovecii	2	4	38.45
H. influenzae	1	2	38.51
RSV (A and B)	1	2	34.71

DISCUSSION

The study highlighted the distinction between co-pathogen detection and true co-infection. Co-pathogen detection refers to the simultaneous identification of various respiratory pathogens, which may not necessarily indicate active infection or interaction between pathogens (Maltezou *et al*., 2023). This distinction is crucial as it impacts clinical management decisions, especially in interpreting the significance of detected pathogens in the context of patient symptoms and outcomes. Biomarkers such as C-reactive protein (CRP) and procalcitonin levels were assessed to provide additional insights into the severity and nature of infections, aiding in distinguishing between colonisation and active infection (Nargis *et al*., 2014).

The findings also indicated a low incidence of respiratory virus co-infections, with only one patient testing positive for RSV, consistent with broader trends reported in other studies (Krumbein *et al*., 2023). This aligns with the observation that COVID-19 primarily affects the respiratory tract, and co-infections or secondary infections typically involve respiratory co-pathogens rather than systemic spread to the bloodstream (Patton *et al*., 2023). The study's use of molecular diagnostics allowed for precise detection and quantification of pathogens, providing valuable data on the prevalence and types of co-detected respiratory pathogens in critically ill COVID-19 patients.

The advancement of nucleic acid amplification techniques (NAAT) has enabled multiplexing to detect multiple bacterial, viral, and fungal pathogens in patients with similar, often non-specific symptoms. This is known as syndromic panel. In the detection of viral respiratory infection, NAAT has replaced viral culture and immunofluorescence as the method of choice. Multiplexed respiratory molecular panel has superior sensitivity, rapid turnaround time, and the ability to simultaneously detect multiple pathogens, with the potential to reduce the length of stay, shorten the duration to targeted antimicrobial therapy, and reduce the number of laboratory tests needed.

Another advantage of such syndromic panel is the ability to detect co-infections of multiple pathogens. However, the detection of one or more respiratory pathogens needs to be interpreted with caution. First, some of the respiratory pathogen detected in the panel, for example *H. influenzae* and *M. catarrhalis*, are part of the commensals of upper respiratory tract (Giufrυ *et al*., 2015). *S. pneumoniae* may also colonize the upper respiratory tract. Specimen collected from the upper respiratory tract using nasopharyngeal swab may contain such organisms. This is particularly so in the paediatric populations (Blaschke, 2011). Second, highly sensitive PCR assay may detect the genetic materials of the pathogen in respiratory secretions for several weeks or more, even after the cessation of the shedding of living organism. Interpretation in such cases may be difficult. In conjunction with the clinical manifestations, the use of quantitative molecular test or semiquantitative results such as the cycle threshold may be of value to indicate the predominant pathogen that may be considered clinically significant. Further studies may be required to establish the threshold to distinguish colonization or contamination versus true infection. Thirdly, as molecular tests are highly sensitive therefore prone to false positive due to cross contamination, a meticulous approach for prevention of such event should be routinely observed in the workflow of the laboratory.

CONCLUSION

Critically ill COVID-19 patients, especially those on corticosteroids and mechanically ventilated, are also susceptibility to other respiratory infections. Syndromic approach is to respiratory infection offers advantages to detect such infections with other respiratory pathogens, rapid turnaround time and superior sensitivity. However, the use of syndromic panel should be selective and guided by patients' clinical presentation and radiological findings, as well as quantitative molecular data, if available, to enable proper interpretation and positively impact the management.

ACKNOWLEDGEMENT

This work was supported by the Universiti Sains Malaysia (USM) Short Term Grant (Grant Number: 304/PPSP/6315459) and the Ministry of Science, Technology, and Innovation (MOSTI) under the Program Strategik Memperkasa Rakyat dan Ekonomi (PEMERKASA) (Grant Number: 305/PPSP/614504).

Conflicts of Interest

All the authors have declared that there is no conflict of interest regarding the publication of this paper. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/COVID19-44 on 19 July 2020).

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