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Genetic Variant of SARS-CoV-2 Isolates in Indonesia: Spike Glycoprotein Gene

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Abstract

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a novel coronavirus and the primary causative agent of coronavirus disease 2019 (COVID-19), first occurred in China and rapidly spread worldwide. The government of the Republic of Indonesia confirmed its first two cases of COVID-19 in March 2020. COVID-19 is a serious illness with no efficacious antiviral medication or approved vaccine currently available. Therefore, there is a need to investigate the genome of SARS-CoV-2. In this study, we characterized SARS-CoV-2 spike glycoprotein genes from Indonesia to investigate their genetic composition and variability. Overall, ten SARS-CoV-2 spike glycoprotein gene sequences retrieved from GenBank (National Center for Biotechnology Information, USA) and the GISAID EpiCoV database (Germany) were compared. We analyzed nucleotide variants and amino acid changes using Molecular Evolutionary Genetics Analysis (MEGA) X and analyzed gene similarity using the LALIGN web server. Interestingly, we revealed several specific mutation sites, however, there were no significant changes in the genetic composition of SARS-CoV-2 spike glycoprotein genes, when compared to the Wuhan-Hu-1 isolate from China. However, this is a preliminary study and we recommend that molecular epidemiology and surveillance programs against COVID-19 in Indonesia be improved.

Keywords: Coronavirus, COVID-19, Genetic composition, Mutation, SARS-CoV-2

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INTRODUCTION

The Chinese government first reported a novel pneumonia-causing disease in Wuhan in December 2019[1]. The causative agent was identified and named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV)[2]. This new virus has rapidly spread across China and to many other countries across the world, including Indonesia[3-4]. The World Health Organization has named the illness caused by SARS-CoV-2 as coronavirus disease 2019 (COVID-19)[5].

According to an online interactive dashboard hosted by the Center for Systems Science and Engineering at Johns Hopkins University (Baltimore, USA), which tracks reported cases of COVID-19 in real-time[6], more than 4 million people have been infected by SARS-CoV-2 worldwide, with more than 14 000 cases in Indonesia alone. Currently, there are three coronaviruses that cause illness in humans: severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2[7].

Taxonomically, coronaviruses belong to the *Coronaviridae* family in the order *Nidovirales*, with examples in four distinct genera: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus*[8]. The structural proteins are encoded by four genes, specifically the envelope (E), nucleocapsid (N), membrane (M), and spike glycoprotein (S)[7,9-10]. Previous studies have shown that the spike glycoprotein plays a crucial role in binding to receptors on the host cell. Therefore, this protein is a key target for a number of antiviral therapies and a promising antigen for generating vaccines formulated against SARS-CoV, MERS-CoV, and SARS-CoV-2.

Molecular epidemiology research is a crucial tool in the surveillance of newly emerging and reemerging viruses[11-12]. Indonesia was the eighth country in Southeast Asia after Brunei, Cambodia, Malaysia, Myanmar, Singapore, Thailand, and Vietnam to report the whole-genome sequences of SARS-CoV-2 in the region. Both Callaway (2020) and Shang *et al*. (2020) have shown that vaccines are being developed against SARS-CoV-2 by various research groups worldwide[13-14]. Similarly, Al-Tawfiq (2020) has discussed other potential therapeutic options for COVID-19[15] and both remdesivir and chloroquine are capable of effectively inhibiting SARS-CoV-2 in *in vitro* assays[16]. Despite these promising treatment options, COVID-19 remains a serious disease with no proven effective antiviral medication or approved vaccine available. Therefore, there is an urgent need to investigate the genome of SARS-CoV-2. In this study, we characterized SARS-CoV-2 spike glycoprotein genes from Indonesia in order to investigate their genetic composition and the similarity between different gene isolates.

MATERIALS AND METHODS

SARS-CoV-2 Isolates

SARS-CoV-2 spike glycoprotein gene (3822 bp) sequences were obtained from GenBank (National Center for Biotechnology Information, USA) and the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV database (Germany) (Table 1).

Genetic Composition Analysis

We analyzed the genetic composition of SARS-CoV-2 spike glycoproteins (both nucleotide variants and amino acid changes) using Molecular Evolutionary Genetics Analysis (MEGA) X[12,17]. We used the Wuhan-Hu-1 isolate as a reference gene, according to Sekizuka *et al*. (2020)[18].

Similarity Analysis

We analyzed the similarity of SARS-CoV-2 spike glycoprotein genes using the LALIGN web server (The SIB Swiss Institute of Bioinformatics, Switzerland) with an E-value threshold of 10.0. The LALIGN program is based on an algorithm first described by Huang and Miller[19].

RESULTS AND DISCUSSION

Coronaviruses infect both animals and humans and lead to various illnesses, including neurological, enteric, and respiratory diseases. There are four distinct genera of coronaviruses: *Alphacoronavirus, Betacoronavirus, Gammacoronavirus,* and *Deltacoronavirus*[8]. SARS-CoV, MERS-CoV, and SARS-CoV-2 are three highly pathogenic coronaviruses, capable of infecting humans, that emerged in 2002, 2012, and 2019, respectively[3-4]. In this study, the genetic compositions and sequence similarities of nine Indonesian SARS-CoV-2 spike glycoprotein genes

Table 1. SARS-CoV-2 isolates obtained from the GenBank and GISAID EpiCoV databases

No	Accession ID	Virus Name	Origin	Submitting Institution	Host	Specimen Source	Coverage
1	MN908947.3 (Reference)	Wuhan-Hu-1	China (Wuhan)	Shanghai Public Health Clinical Center and School of Public Health, Fudan University, Shanghai	*Homo sapiens*	Unknown	Reference genome
2	EPI_ISL_435281	JKT-EIJK0141	Indonesia (Jakarta)	Eijkman Institute for Molecular Biology, Ministry of Research and Technology/ National Agency for Research and Innovation of the Republic of Indonesia	*Homo sapiens*	Nasopharyngeal and Oro-pharyngeal swab	22×
3	EPI_ISL_435282	JKT-EIJK0317	Indonesia (Jakarta)	Eijkman Institute for Molecular Biology, Ministry of Researchand Technology/ National Agency for Research and Innovation of the Republic of Indonesia	*Homo sapiens*	Nasopharyngeal and Oro-pharyngeal swab	1,480×
4	EPI_ISL_435283	JKT-EIJK2444	Indonesia (Jakarta)	Eijkman Institute for Molecular Biology, Ministry of Research and Technology/ National Agency for Research and Innovation of the Republic of Indonesia	*Homo sapiens*	Nasopharyngeal swab	8,082×
5	EPI_ISL_437187	EJ-ITD853Sp	Indonesia (Surabaya)	Institute of Tropical Disease, Universitas Airlangga	*Homo sapiens*	Sputum	764×
6	EPI_ISL_437188	EJ-ITD3590NT	Indonesia (Surabaya)	Institute of Tropical Disease, Universitas Airlangga	*Homo sapiens*	Nasopharyngeal and Oro-pharyngeal swab	96×
7	EPI_ISL_437189	JKT-EIJK01	Indonesia (Jakarta)	Eijkman Institute for Molecular Biology, Ministry of Research andTechnology/ National Agency for Research and Innovation of the Republic of Indonesia	*Homo sapiens*	Nasopharyngeal and Oro-pharyngeal swab	2,256×
8	EPI_ISL_437190	JKT-EIJK02	Indonesia (Jakarta)	Eijkman Institute for Molecular Biology, Ministry of Research and Technology/ National Agency for Research and Innovation of the Republic of Indonesia	*Homo sapiens*	Nasopharyngeal swab	5,297×
9	EPI_ISL_437191	JKT-EIJK03	Indonesia (Jakarta)	Eijkman Institute for Molecular Biology, Ministry of Research and Technology/ National Agency for Research and Innovation of the Republic of Indonesia	*Homo sapiens*	Nasopharyngeal swab	2,112×
10	EPI_ISL_437192	JKT-EIJK04	Indonesia (Jakarta)	Eijkman Institute for Molecular Biology, Ministry of Research and Technology/ National Agency for Research and Innovation of the Republic of Indonesia	*Homo sapiens*	Nasopharyngeal and Oro-pharyngeal swab	5,759×

Table 2. Nucleotide mutation sites in the SARS-CoV-2 spike glycoprotein

No.	Virus Name	Nucleotide Position							
		224	347	414	1715	1841	1864	2031	2464
1	Wuhan-Hu-1 (Reference)	C	C	T	C	A	G	G	C
2	JKT-EIJK0141	C	C	T	C	A	G	G	C
3	JKT-EIJK0317	C	C	T	C	A	G	G	C
4	JKT-EIJK2444	T	C	T	C	A	G	G	C
5	EJ-ITD853Sp	C	C	T	C	A	G	G	C
6	EJ-ITD3590NT	C	G	T	C	G	G	T	C
7	JKT-EIJK01	C	C	C	C	A	T	G	C
8	JKT-EIJK02	C	C	T	C	A	G	G	C
9	JKT-EIJK03	C	C	T	C	A	G	G	C
10	JKT-EIJK04	C	C	T	T	A	G	G	T

Table 3. Amino acid mutation sites in the SARS-CoV-2 spike glycoprotein

No	Virus Name	Amino Acid Position						
		76	116	572	614	622	677	822
1	Wuhan-Hu-1 (Reference)	T	S	T	D	V	Q	L
2	JKT-EIJK0141	T	S	T	D	V	Q	L
3	JKT-EIJK0317	T	S	T	D	V	Q	L
4	JKT-EIJK2444	I	S	T	D	V	Q	L
5	EJ-ITD853Sp	T	S	T	D	V	Q	L
6	EJ-ITD3590NT	T	C	T	G	V	H	L
7	JKT-EIJK01	T	S	T	D	F	Q	L
8	JKT-EIJK02	T	S	T	D	V	Q	L
9	JKT-EIJK03	T	S	T	D	V	Q	L
10	JKT-EIJK04	T	S	I	D	V	Q	F

were determined using sequences obtained from GenBank and the GISAID EpiCoV database (Table 2-4). Many researchers worldwide have previously reported mutations in the SARS-CoV-2 genome[10,20-21], however, there has not previously been a study of the SARS-CoV-2 genome from Indonesian isolates.

The coronavirus spike glycoprotein mediates membrane fusion and viral entry into host cells and is therefore the primary target for many neutralizing antibodies (Fig. 1). The spike glycoprotein has two domains, S1 and S2, where S1 is responsible for binding the virion to ACE2 on the host cell membrane[21]. Several antiviral drugs and vaccines have been developed which target the spike glycoprotein. Du *et al*. (2009)[22] demonstrated the effectiveness of several of these antiviral therapies, including small interfering RNAs, protease inhibitors, ACE2 blockers, fusion blockers, spike glycoprotein inhibitors, neutralizing antibodies, and spike glycoprotein cleavage inhibitors in *in vitro* studies. In addition, a number of techniques have been used to generate vaccines using all or part of the spike glycoprotein as an antigen. These include the use of recombinant receptor binding domain protein, viral vectors, full-length S protein, recombinant spike glycoprotein, and spike protein DNA-expressing vectors. Thus, it is very important to investigate the genetic composition and sequence similarity of this protein.

Interestingly, despite reporting several mutation sites in this study, we demonstrate that there is no significant change in the genetic composition of SARS-CoV-2 spike glycoprotein genes. Nucleotide variants in SARS-CoV-2 spike

Table 4. Sequence similarity of SARS-CoV-2 spike glycoprotein genes, determined using the LALIGN web server

Virus Name	Similarity								
	Wuhan-Hu-1 (Reference)	JKT-EIJK0141	JKT-EIJK0317	JKT-EIJK2444	EJ-ITD853Sp	EJ-ITD3590NT	JKT-EIJK01	JKT-EIJK02	JKT-EIJK03
JKT-EIJK0141	100%								
JKT-EIJK0317	100%	100%							
JKT-EIJK2444	100%	100%	100%						
EJ-ITD853Sp	100%	100%	100%	100%					
EJ-ITD3590NT	99.9%	99.9%	99.9%	99.9%	99.9%				
JKT-EIJK01	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%			
JKT-EIJK02	100%	100%	100%	100%	100%	99.9%	99.9%		
JKT-EIJK03	100%	100%	100%	100%	100%	99.9%	99.9%	100%	
JKT-EIJK04	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%

glycoprotein genes described in this study include JKT-EIJK2444 (224C>T), EJ-ITD3590NT (347C>G; 1841A>G; 2031G>T), JKT-EIJK01 (414T>C; 1864G>T), and JKT-EIJK04 (1715C>T; 2464C>T) (Table 2). In addition, we also analyzed amino acid changes in the SARS-CoV-2 spike glycoprotein including JKT-EIJK2444 (T76I), EJ-ITD3590NT (S116C; D614G; Q677H), JKT-EIJK01 (V622F), and JKT-EIJK04 (T572I; L822F) (Table 3). Finally, the interval score of similarity between each of the SARS-CoV-2 spike glycoproteins was between 99.9% and 100% (Table 4).

Previous studies investigating gene variability in SARS-CoV-2 samples include the work of Sekizuka *et al*. (2020), who performed whole-genome sequencing of SARS-CoV-2, directly from PCR-positive clinical specimens[18]. This was conducted in order to generate a haplotype network analysis of the Diamond Princess cruise ship outbreak, using the Wuhan-Hu-1 sequence as a reference. Additionally, Castillo *et al*. (2020) reported a phylogenetic analysis of the first four SARS-CoV-2 cases in Chile, analyzing nucleotide variants, amino acid changes, and sequence similarity[23]. While our study complements these previous works, several shortcomings remain, including the relatively small number of isolates studied, the methodology used for whole-genome sequencing, and the quality coverage of SARS-CoV-2 genomes isolated from Indonesia.

As new information on SARS-CoV-2 is published daily, new concepts and frameworks must constantly be adopted. Currently, the GISAID EpiCoV database and Tang *et al*. (2020) have established three subtypes of SARS-CoV-2 based on nucleotide variants that produce amino acid changes: S, G, and V[24]. The mutation rate of viruses is considerably higher than most other biological entities, including prokaryotes and eukaryotes. This is especially true of RNA-based viruses such as SARS-CoV-2, Ebola and dengue, due to hydroxyl groups in RNA that act as catalytic sites for mutation. This advanced mutation rate, leads to enhanced virulence and a higher capacity for adaptive evolution[25-26]. While Tang *et al*. (2020) have suggested that SARS-CoV-2 exhibits the characteristic high mutation rate of an RNA virus[24], in fact, the mutation rate of SARS-CoV-2 and other coronaviruses might be slightly lower than other RNA viruses because of its genome-encoded

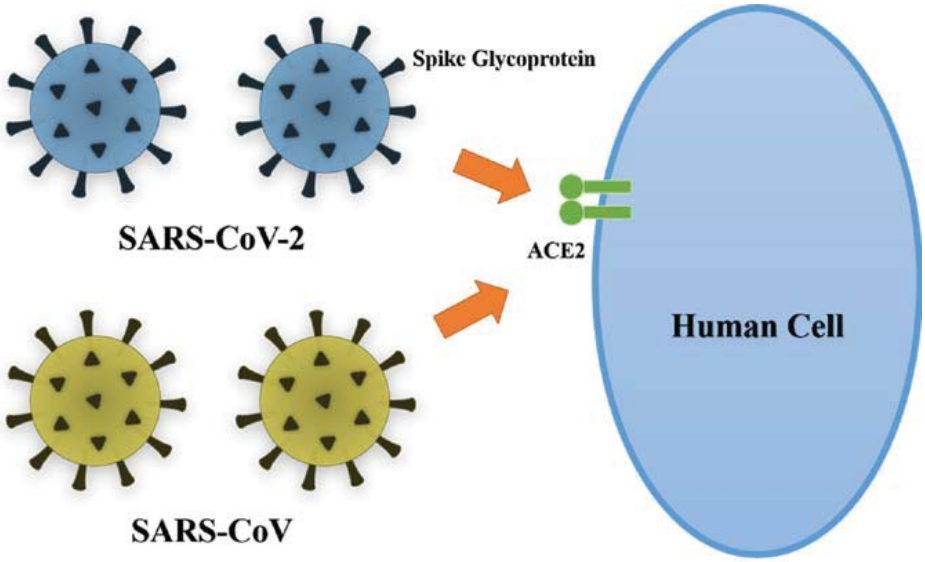

Fig. 1. Schematic diagram of SARS-COV and SARS-CoV-2 binding with high affinity to ACE2, an essential step in viral entry into host cells.

exonuclease. Regardless, its high mutation rate increases the potential of this zoonotic viral pathogen to adapt to efficient transmission from human to human and potentially allows it to become more virulent.

The genomic characteristics of SARS-CoV-2 are significantly different than either SARS-CoV or MERS-CoV[27]. A previous study reported that the homology of SARS-CoV-2 with the bat coronavirus isolate, RaTG13, was 96%[28]. Interestingly, another study reported that the homology of SARS-CoV-2 with a pangolin coronavirus was 99%[1]. From these results, it could be suggested that pangolins act as an intermediate host between bats and humans.

This study of genomic variants of SARS-CoV-2 isolated from Indonesia is crucial for future investigations into the pathogenesis, prevention, and treatment of SARS-CoV-2. Development of this genomic data is vital work that will facilitate vaccine design, epidemiological investigations, viral detection, functional analysis, and evaluation of treatment options[27].

Outbreaks of SARS-CoV-2 have led to a state of medical and economic emergency worldwide. Therefore, understanding the characteristics of the SARS-CoV-2 genome and developing systems to monitor SARS-CoV-2 during the pandemic are critical steps for controlling this disease. The identification of genotypes connected to specific geographic and temporal infectious clusters suggests that genomic data can be used to track and monitor the transmission of SARS-CoV-2. Therefore, the rapid discovery of genetic variants of SARS-CoV-2 is necessary for a streamlined response to the COVID-19 outbreak. Similarly, identifying specific SARS-CoV-2 variants and connecting them using a molecular epidemiology approach would allow researchers to determine the origin of a specific variant and monitor its transmission. This could be an important tool in controlling the outbreak[21].

CONCLUSION

In summary, there was no significant difference between the SARS-CoV-2 spike glycoprotein gene sequences found in Indonesia and the Wuhan-Hu-1 isolate from China. However, this was only a preliminary study and we recommend expanding molecular epidemiology and surveillance programs to monitor COVID-19 in Indonesia.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All listed authors made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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ETHICS STATEMENT

This article does not contain any experiments using human participants or animals performed by any of the authors.

AVAILABILITY OF DATA

Not applicable.

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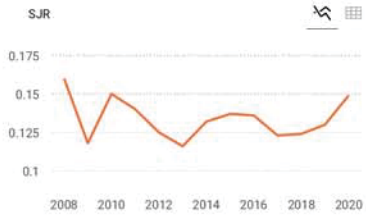

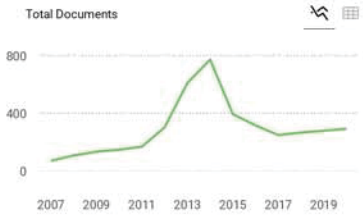

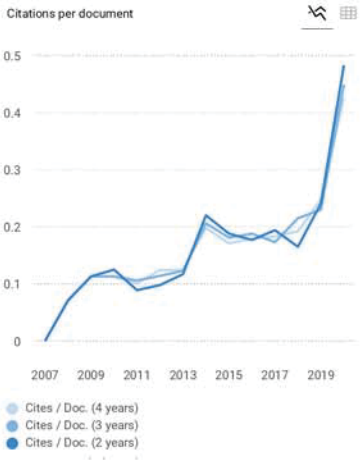

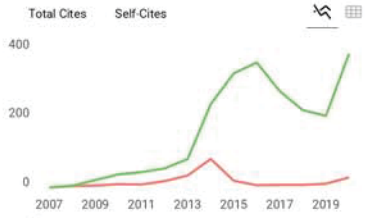

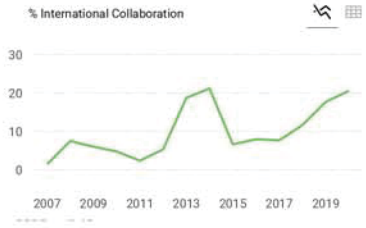

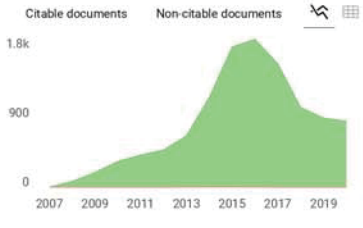

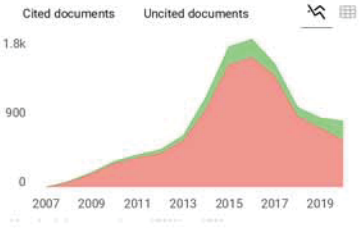

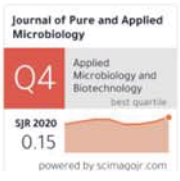

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Manuscript send at the end of Feb 2020.

reply

SCImago Team

Melanie Ortiz 1 year ago

Dear Ravindra,
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Y **Yermia** 2 years ago

Whether the journal is still indexed in scopus ?

reply

SCImago Team

Melanie Ortiz 2 years ago

Dear Yermia, thank you very much for your comment, unfortunately we cannot help you with your request. We suggest you consult the Scopus database directly. Keep in mind that the SJR is a static image (the update is made one time per year) of a database (Scopus) which is changing every day.

R **Rittishai saelim** 2 years ago

This journal is now included in the updared Beall's list of predatory journals. Could anyone verify the validity of this journal please?

reply

D **Dr. M. N. Khan** 2 years ago

Dear Rittishai,
Our journal had-been removed from the Beall's list of potential predatory journals and publishers in their last update dated June 07, 2020. For your reference, you can check it here: https://beallslist.net/

For any further information, please feel free to contact us.
Best Regards
Dr. M. N. Khan
Editor
Journal of Pure and Applied Microbiology
www.microbiologyjournal.org

D **Dr. M. N. Khan** 2 years ago

Dear Rittishai,

Thanks for your comment. Kindly note, the current Beall's list (https://beallslist.net/) is not updated from December 28, 2019. Also, our journal was not in the Original predatory list created by the librarian Mr. Jeffrey Beall. We have already tried to reach through the contact form couple of times to the anonymous person currently maintaining Beall's list but unfortunately, we haven't received any reply from their side. Our journal is indexed in Scopus (Elsevier), Emerging Sources Citation Index (Web of Science, Clarivate Analytics), DOAJ (Directory of Open Access Journals), CAB Abstracts (CABI), etc.

Moreover, you can read it yourself (https://beallslist.net/how-to-recognize-predatory-journals/) clearly mentioned on the current Beall's list website that if a journal is indexed in JCR or/and DOAJ, that is usually a very good indicator that the journal is not predatory.

For any further information, please feel free to contact us at: editor@microbiologyjournal.org

Thanks and Regards

Dr. M. N. Khan
Editor
Journal of Pure and Applied Microbiology
www.microbiologyjournal.org/

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Melanie Ortiz 2 years ago

Dear Rittishai,
thank you very much for your comment, unfortunately we cannot help you with your request. We suggest you contact directly with Scopus support: https://service.elsevier.com/app/answers/detail/a_id/14883/kw/scimago/supporthub/scopus/
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B **Bayar Zeebaree** 2 years ago

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Melanie Ortiz 2 years ago

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A **Abdullahi Balarabe Inuwa** 3 years ago

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Regards,
Abdullahi Balarabe Inuwa

reply

A **Azim Aijaz** 3 years ago

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If you have any further query feel free to write us on, editor@microbiologyjournal.org

Thanks and Regards

Azim Aijaz

SCImago Team

Elena Corera 3 years ago

Dear Abdullahi Balarabe Inuwa,

we are so sorry, but we cannot help you with your request. We are an institution absolutely different from the journals, so we cannot provide you any information different from what you can find in their websites.
Please, contact journal editorial staff so they could solve your request.

Best regards,
SCImago Team

F **Fatalla** 3 years ago

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I would like to know if the journal continue with scopus coverage in this year 2018
Regards

reply

SCImago Team

Elena Corera 3 years ago

Dear Fatalla,
thank you for your request, all the journals included in SJR are indexed in Scopus. Elsevier / Scopus is our data provider.
Best Regards,
SCImago Team

A **Ameer** 3 years ago

Thanks Elena for this information

reply

J **Jenni** 3 years ago

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reply

J **Jenni** 3 years ago

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reply

A **Azim Aijaz** 3 years ago

Dear Jenni,

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Kindly visit the following link to download your paper.

https://microbiologyjournal.org/archive/
If you have any further query feel free to write us back.

SCImago Team

Elena Corera 3 years ago

Corrected, thanks!!!

N **Nermin Şimşek Kuş** 3 years ago

Dear editor,

I sent an article ("The synthesis of two novel bicyclic haloketones and the measurement of their biological activity") in May. But no answer came. Would you give me information about the article.

Prof. dr. Nermin Şimşeek Kuş
simner@mersin.edu.tr

reply

Elena Corera 3 years ago

SCImago Team

Dear Nermin, we suggest you contact the journal directly. Best Regards, SCImago Team

N **nihad** 3 years ago

good evening :
I would like to publish my research in your journal

reply

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Iqbal Ahmad is currently Professor of the Department of Agricultural Microbiology at Aligarh Muslim University in Aligarh, India. He received his Ph.D. degree from Aligarh Muslim University in Agriculture Microbiology, during his Ph.D. research work he worked at the Division of Microbiology, CSIR-Central Drug Research Institute, Lucknow, India with pioneer veterinary bacteriologist, Late Dr. JNS Yadava, FNA, FNASc. He first worked as a Research Scientist at The Himalya Drug Co., New Delhi in 1994 and then joined as a Lecturer at the Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh in 1995 and then promoted to Professor in 2010. He was the Section Incharge of Microbiology during 1999-2000 and has also chaired the Department during 2011-2013 and in 2017. He has done pioneering research on plasmid mediated drug resistance and virulence factors in enteric bacteria and strategies to combat drug resistance and virulence of pathogenic microbes through screening and evaluation of Indian medicinal plant derived herbal products/extracts and phytocompounds especially targeting Quorum sensing, biofilm and virulence of pathogenic microorganisms. His research work on agriculturally important microorganisms on diazotrophs, biofilm forming PGPR and impact of wastewater on soil health has been well documented. His recent interest on interdisciplinary microbiological works and drug-macromolecule interactions are gaining importance in academic world.

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