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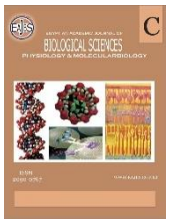
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 16(2) pp 1-16 (2024)*
DOI: 10.21608/EAJBSC.2024.363885

Egypt. Acad. J. Biolog. Sci., 16(2):1-16 (2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Delineation of Genomic Variants/Covariants of SARS-CoV-2, Their Frequencies, and Cooccurrence in Saudi Arabia in Context with Neighboring Countries

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ARTICLE INFO
Article History
Received:9/5/2024
Accepted:4/7/2024
Available:8/7/2024

Keywords:
Variation, spike, outbreak, COVID-19, CooPer.

ABSTRACT

Background: Accumulating remarkable variations in spike-gene (Spk-gene) contribute to the evolution of potential spike (Spk)-variants and covariants of SARS-CoV-2 which influence prophylactic, diagnostic, and therapeutic countermeasures**. Objective:** The current research was designed to delineate the SARS-CoV-2 Spike-variant (Spk-variant) and SARS-CoV-2-Spike-covariant (Spk-covariant) frequencies and their cooccurrences in Saudi Arabia and the geographical regions around it. **Methods:** Relevant data **(**SARS-CoV-2-Spk-variant and SARS-CoV-2-Spk-covariants RNA-genome-sequences) offered by Bacterial and Viral Bioinformatics Resource Center (BV-BRC) was mined and retrieved using SARS-CoV-2-variants-tracker (real-time-based). Execution of comparative analysis of the data was accomplished using tidyverse/png/grid of the R-packages. Query datasets were generated for KSA and other neighboring regions and analyzed with cooccurrence and persistence network-clustering algorithms based on R-base-R packages and libraries. Data visualization was carried out by employing ggplot2/cooccurrence plots. **Results:** The substitution N501T, F157S, and H49Y in KSA, P681H in Iraq, and T478K in Bahrain were the most frequent variants. The Spk-variant-B.1, B.1.1.7, A.28, B.1.36 in KSA, CP.1, BA.2.75.2, BE.3, BM.1.1.3 in Qatar, XBB.1, EG.1in Kuwait, B. 1 in Jordan, B.1.1.7, B.1.428.1 in Iraq, and BA.1 and B.1.1.7 in Bahrain were the most frequent covariants. The Spk-variants (Spk-variant-N501Y and P681H) cooccurred in KSA and the majority of the neighboring countries (n = 6). **Conclusion:** The outcome of this research could be leveraged for deciding the appropriate prophylactic, diagnostic, and therapeutic countermeasures for the effective management of future outbreaks of disease in these regions.

INTRODUCTION

SARS-CoV-2-lineage-switching and generating several variants/covariants at higher frequencies (Harvey *et al.* 2021) contribute enormously to regional and global transmission, causing gross health concerns and posing economic setbacks for healthcare (Wang *et al.* 2023). The COVID-19 caused approximately seven million deaths over 3 years (Jaumdally *et al.* 2024). SARS-CoV-2 is a lethal virus for humans causing severe infection at the pulmonary level and beyond (Chen *et al.* 2020; Tabibzadeh *et al.* 2020). End of December, 20019, this virus with greater potential to transmit was reported initially from Wuhan (Linka *et al.* 2020; Parlikar *et al.* 2020; Tan *et al.* 2020).

Plus (+) RNA (30kb length) constitutes the genome of the virus encoding structural-proteins (SPs), open-reading-frames/ORFs:accessory-proteins (ORF: APs), and non-structural-proteins (NSPs) (Brant *et al.* 2021; Chan *et al.* 2020; Rohaim *et al.* 2021). The SARS-CoV-2's genome/RNA replicates with a greater rate of mutation due to the error-prone replication enzyme (RNA-polymerase-enzyme) (Su *et al.* 2016). The variation frequency in the viral genome was assessed to be lesser in comparison to the other viruses having ssRNA genomes (Amicone *et al.* 2022; Callaway 2020). However, the greater adaptability of the Spk-variants to the host environment and its large-scale-transmissibility affecting vast geographic regions rendered to encompass a huge mutational pile-up, particularly in the most significant structural protein (spike) besides other SPs and NSPs (Telenti *et al.* 2021).

Accumulation of several S-gene-mutation constitutes the basis of the generation of variants, covariants, lineages, and SARS-CoV-2:sub-lineages with varying infection dynamics including infectivity, transmissibility, host immune response, and pathogenicity regionally and worldwide (Volz *et al.* 2021). Furthermore, newly evolved Spk-covariants/Spk-variants gain the capability for immune evasion triggered by natural active or artificial active immunization (Edara *et al.* 2021; Xiaoying Shen *et al.* 2021; Wang *et al.* 2021). Besides the evolution/emergence of viral-Spk-VOIs, for instance, Iota, Eta, Kappa, Epsilon, Lambda, and Zeta, several significant SARS-CoV-2-Spk-VOC (Spk-Omicron, Spk-Alpha/B.1.1.7, Spk-Beta/B.1.351, Spk-Gamma/P.1, and Spk-Delta/B1.617.2) have been recognized which affect the global population severely (Ortiz-Gómez *et al.* 2023; Zhang *et al.* 2022). VOCs have the potential to alter the COVID-19 disease-associated epidemiological parameters (Kumar *et al.* 2022). Compared to the wild-type-strain/phenotype or initially evolved Spk-variants, the newly emerged Spk-variants/Spk-covariants may trigger fresh waves of COVID-19 disease with less understood epidemiological characteristics in any region including Saudi Arabia (Brookman *et al.* 2021; Kustin *et al.* 2021).

In addition, VOCs emergence affects the effective implementation of health-safety policies, and social measures taken to curb outbreaks besides therapeutic, and diagnostic countermeasures (Zhang *et al.* 2022). Additionally, the frequency of the variants or Spk-covariants in specific geography influences the transmissibility, virulence (Malik 2022), and effectiveness of vaccination in these areas (Rabaan *et al.* 2023). Furthermore, the regional importation (from/to neighboring countries) of the Spk-variants and Spk-covariants and within-country spread can influence the dynamics of the control measures taken to curb the outbreak regionally (Han *et al.* 2022). Therefore, the delineation of Spk-variants/Spk-covariants becomes crucial for the efficient management of the outbreak (McLean *et al.* 2022; Wang *et al.* 2021) necessitating the tracking of the variants and covariants and their regional magnitude (frequency) to assess the right choice of therapeutic intervention. The current study aimed to execute analyses of Spk-variants and Spk-covariants and their frequencies in Saudi Arabia and its neighboring countries. From these analyses, understandings of prevalent Spk-variants and Spk-covariants in these regions were deduced that could be leveraged to understand the genomic variation, importation from neighboring countries to Saudi Arabia, and viral evolution. The findings of this research can be used to assess the appropriate vaccine, social measures, effective regional outbreak management, and the necessity of the multivalent vaccine.

MATERIALS AND METHODS

1-Data Acquisition, Resources, and Quality Assurance:

The relevant data for the present study was mined and acquired from the Bacterial and Viral Bioinformatics-Resource-Center (BV-BRC) harboring information

suitable for executing research on viral pathogens including SARS-CoV-2-virus (Bacterial and Viral Bioinformatics Resource-Center | BV-BRC). Data on Spk-variants and Spk-covariants was acquired and plugged into an Excel sheet to generate different data sets for variant/covariant analyses, comparison, and visualization. Incomplete, redundant, and repeated information was excluded during data cleaning to avoid analytical errors. The vital components of the analytical methods and resources are illustrated in Figure 1.

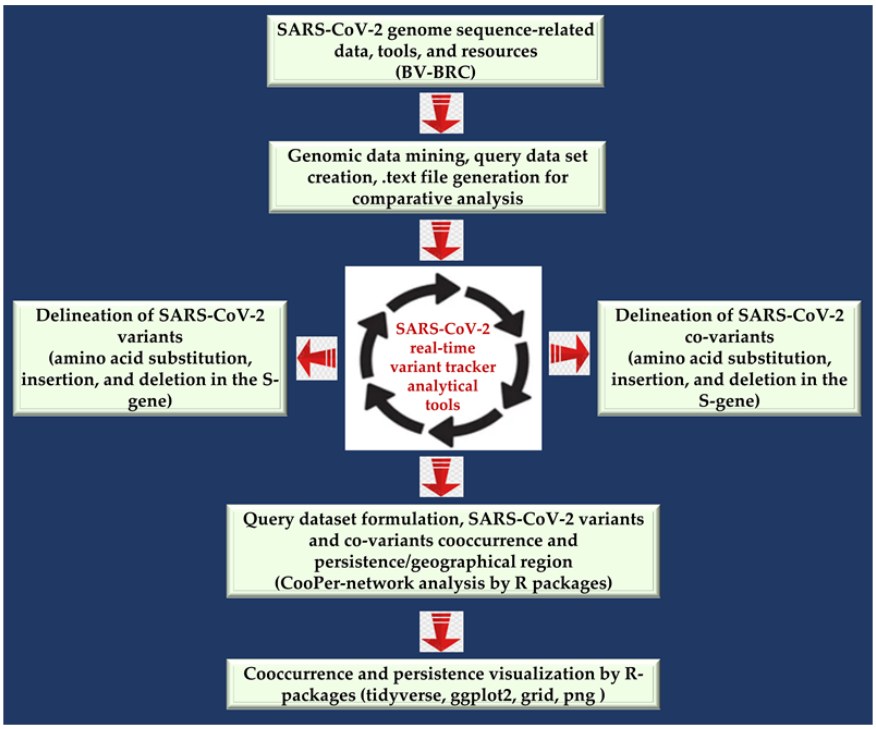

Fig. 1. Demonstration of the execution methods, analytical tools, libraries, and packages employed in the study. BV-BRC = Bacterial and Viral Bioinformatics Resource Center and CooPer = cooccurrence and persistence.

2-Analytical Techniques and Processes:

For comparative variant and covariant analysis, SARS-CoV-2-virus-variants-trackers offered at tools and services-BV-BRC tabs were employed (https://www.bvbrc.org/view/VariantLineage). The Spk-Variants and Lineages-of-Concern-Resource (LoCR) were leveraged for the delineation of emerging Spk-variants, Spk-covariants, and SARS-CoV-2-lineages specific geography of interest (Saudi Arabia, Kuwait, Qatar, Bahrain, Jordan, UAE, Oman, and Yemen). The output of the SARS-CoV-2-Real-time Tracking and Early-Warning-System for tracking Spk-variants and Lineages-of-Concern/Lineage-of-Interst(VoCs/LoCs) is dependent on regular processing of the genomic data (publicly available) of Spk-variants/Spk-covariants. The listing of VOCs and VoIs was done based on the Centers for Disease Control and Prevention (CDC) and the National Institute of Allergy and Infectious Disease (NIAID). Relevant data relating to the common variants and covariants among Saudi Arabia and neighboring countries were mined to compare the frequencies of a particular variant or co-variants by plotting the variants/co-variants related data by using several packages of the R-studio. The neighboring countries without any reported Spk-variants and Spk-covariants were not included in the current research.

3-Operational Definition:

Wuhan-hu-1- ref-genome (NC_045512.2) was taken as a standard to characterize all the variants in the study (Wu

et al. 2020). The definition of the Spk-variants was based on the variation in the consensus sequence of the Spk-gene (S-gene) region compared to that of the reference genome. The variations were characterized as amino acid substitution (amino acid residue in reference genome followed by a location in Spk-gene followed by amino acid residues in VOCs/LOCs/VoIs), deletion (amino acid residue in reference genome followed by a location in Spk-gene), insertion (location in Spk-gene of lineage followed by amino acid residues in VOCs/LOCs/VoIs) (Akaishi 2022).

4-Cooccurrence and Persistence of Variants and Covariants:

For determining the co-occurrence and persistence of the variants and Spk-covariants, the CooPer-network analyses were executed by adopting the concept of an R-based algorithm (Varghese *et al.* 2021). For Spk-variants network analysis, the query datasets were generated which had variants information of the n = 6 different geographic regions. For determining the Spk-covariants network analysis, query datasets were generated which contained covariants information of n = 6 different geographical regions. The unclassified variant sequences were excluded from the study during the data-cleaning steps. The cooPer-network plots were deduced to understand the most and the least common variants and covariants persisting and co-occurring in the geographical regions of the current study's interest (Varghese *et al.* 2021).

RESULTS

1-Baseline Features of Spk-Variants by Region:

The Spk-variants (N501T, F157S, H49Y, Y144, T859N, A899S, G257S, H69, V70, P812S, N501Y, V143, Y144D, A570D, D1118H, P681H, S982A, T716I, H655Y, and P330S) reported from Saudi Arabia was observed which included n = 4 deletions and 16 amino acid substitutions. SARS-CoV-2 Spk-variants reported from Qatar were Spk-variants/A27S, D405N, D796Y, G142D, H655Y, K417N, L24, N440K, N501Y, N679K, N764K, N969K, P25, P26, P681H, Q498R, Q954H, R408S, S371F, and S373P with with n = 3 deletions. The Spk-variants observed in Kuwait were Spk-variantsA27S, D405N, D796Y, E484A, G142D, H655Y, K417N, L24, N440K, N460K, N501Y, N679K, N764K, N969K, P25, P26, P681H, Q498R, Q954H, R408S with n = 4 deletions. A total of n= 3 deletions and 9 substitutions were noticed out of variants A899S, D1139Y, G1167S, H69, L176F, L452R, Q677H, R346S, S12F, T547I, V70, W152R, and Y144 in Jordan. P681H, H69, V70, N501Y, Y144, S982A, A570D, D1118H, T716I, T478K, G142D, L452R, A522V, P681R, T19R, N679K, D950N, E156G, E484A, and F157 Spk-variants were assessed in Iraq. Similarly, the Spk-variants were evaluated to be T478K, P681H, H655Y, N679K, N969K, Q954H, D796Y, N764K, S373P, S375F, G142D, N501Y, Y505H, Q498R, S477N, E484A, S371F, T376A, D405N, A27S4A, S371F, T376A, D405N, and A27S in Bahrain (Table 1).

2-Baseline Spk-Covariants Feature Stratified by the Region:

The Spk-covariants observed in Saudi Arabia were Spk-covariants-B.1, B.1.1.7, A.28, B.1.36, B.1.468, C.17, B.1.351, B.1.456. Spk-covariants from Qatar were observed as Spk-covariants-CP.1, BA.2.75.2, BE.3, BM.1.1.3, BA.5.2, BM.1, FL.13, HK.2, XBB, and XBB.1.5.65. The Spk-covariants from Kuwait were reported to be XBB.1, BQ.1.1, EG.1, BE.9, BQ.1, EG.14, FL.2, FL.4, XBB.1.5, XBB.1.5.64, and XBB.1.9.1**.** Very few Spk-covariants (B.1.1, B.1, A.5, B.1.319, B.40, and C.36.3) were observed in Jordan. The Spk-covariants observed from Iraq were B.1.1.7, B.1.428.1, B.1.617.2, B.1.1, B.1, BA.1.1, BA.1, AY.103, AY.33, B.1.36.1, BA.5.2, AY.122, AY.126, B.1.1.529, B.1.177, BA.2, XBB.1, XBB.2, and B. Whereas, the Spk-covariants predominated in Bahrain were BA.2, B.1.617.2, AY.65, BA.5.2, XBB.1, BA.1.1, AY.3.4, BA.2.10, AY.33, BA.1.17.2, BA.5.2.1, AY.112, BN.1, BA.2.3, AY.122, FL.10, FL.5, AY.127, BA.1, and B.1.1.7 (Table 1).

Table 1. Baseline features of the SARS-CoV-2-spike variants and co-variants and the magnitude of their occurrence.

SARS-CoV-2:S-gene: variants and cooccurrence			**SARS-CoV-2:S-gene: covariant and cooccurrence**	
Variants	VT	MOC	Covariant	MOC
A27S	AAS	03	A.28	1
A27S4A	AAS	01	A.5	1
A522V	AAS	01	A.Y103	1
A570D	AAS	2	AY.112	1
A899S	AAS	2	AY.122	2
D1118H	AAS	2	AY.126	1
D1139Y	AAS	1	AY.127	1
D405N	AAS	4	AY.3.4	1
D796Y	AAS	3	AY.33	2
D950N	AAS	1	AY.65	1
E156G	AAS	1	B	1
E484A	AAS	3	B.1	3
F157	AD	1	B.1.1	2
F157S	AAS	1	B.1.1.529	1
G1167S	AAS	1	B.1.1.7	3
G142D	AAS	4	B.1.117	1
G257S	AAS	1	B.1.319	1
H49Y	AAS	1	B.1.351	1
H655Y	AAS	4	B.1.36	1
H69	AD	3	B.1.36.1	1
K417N	AAS	2	B.1.428.1	1
L176F	AAS	1	B.1.456	1
L24	AD	2	B.1.468	1
L452R	AAS	2	B.1.617.2	2
N440K	AAS	2	B.40	1
N460K	AAS	1	BA.1	2
N501T	AAS	1	BA.1.1	2
N501Y	AAS	5	BA.1.17.2	1
N679K	AAS	4	BA.2	2
N764K	AAS	3	BA.2.10	1
N969K	AAS	3	BA.2.3	1
P25	AD	2	BA.2.75.2	1
P26	AD	2	BA.5.2	3
P330S	AAS	1	BA.5.2.1	1
P681H	AAS	5	BE.3	1
P681R	AAS	1	BE.9	1
P812S	AAS	1	BM.1	1
Q498R	AAS	3	BM.1.1.3	1
Q677H	AAS	1	BN.1	1
Q954H	AAS	3	BQ.1	1
R346S	AAS	1	BQ.1.1	1
R408S	AAS	2	C.17	1
S12F	AAS	1	C.36.3	1
S371F	AAS	3	CP.1	1
S373P	AAS	2	EG.1	1
S375F	AAS	1	EG.14	1
S477N	AAS	1	FL.10	1
S982A	AAS	2	FL.13	1
T19R	AAS	1	FL.2	1
T376A	AAS	2	FL.4	1
T478K	AAS	2	FL.5	1
T547I	AAS	1	HK.2	1
T716I	AAS	2	XBB	1
T859N	AAS	1	XBB.1	3
V143	AD	1	XBB.1.5	1
V70	AD	3	XBB.1.5.64	1
W152R	AAS	1	XBB.1.5.65	1
Y114	AD	3	XBB.1.9.1	1
Y114D	AAS	1	XBB.2	1
Y505H	AAS	1	-	-

AAS = amino acid substitution, AD = amino acid deletion, VT = variant-type, MOC = magnitude of the cooccurrence

3-The Status of the Spk-Variants and Spk-Covariants Frequency:

The most frequently identified variants in KSA were Spk-N501T, F157S, and H49Y while Y144, T859N, A899S, and G257S variants were moderately predominant. And the least predominant Spk-variants were H655Y and P330S in KSA (Fig. 2). Moreover, in Iraq, the most predominantly characterized variant was P681H and the H69, V70, and Y144 deletions were highly predominant. Spk-variant-N501Y, S982A, A570D, D1118H, T716I, and T478K were highly prevalent variants (Figure 2). Furthermore, the variants E156G, E484A, and F157 were least frequently identified in Iraq (Figure 2). In addition, the Spk-variant T478K exhibited the highest frequency, while Spk-variant-P681H, H655Y, N679K, N969K, and Q954H were moderately characterized spike-variants in Bahrain and the variants with the least frequency were A27S, T376A, and D405N (Figure 2). The frequency of Spk-variants reported from Qatar, Jordan, and Kuwait was the same/identified variant (Fig. 2).

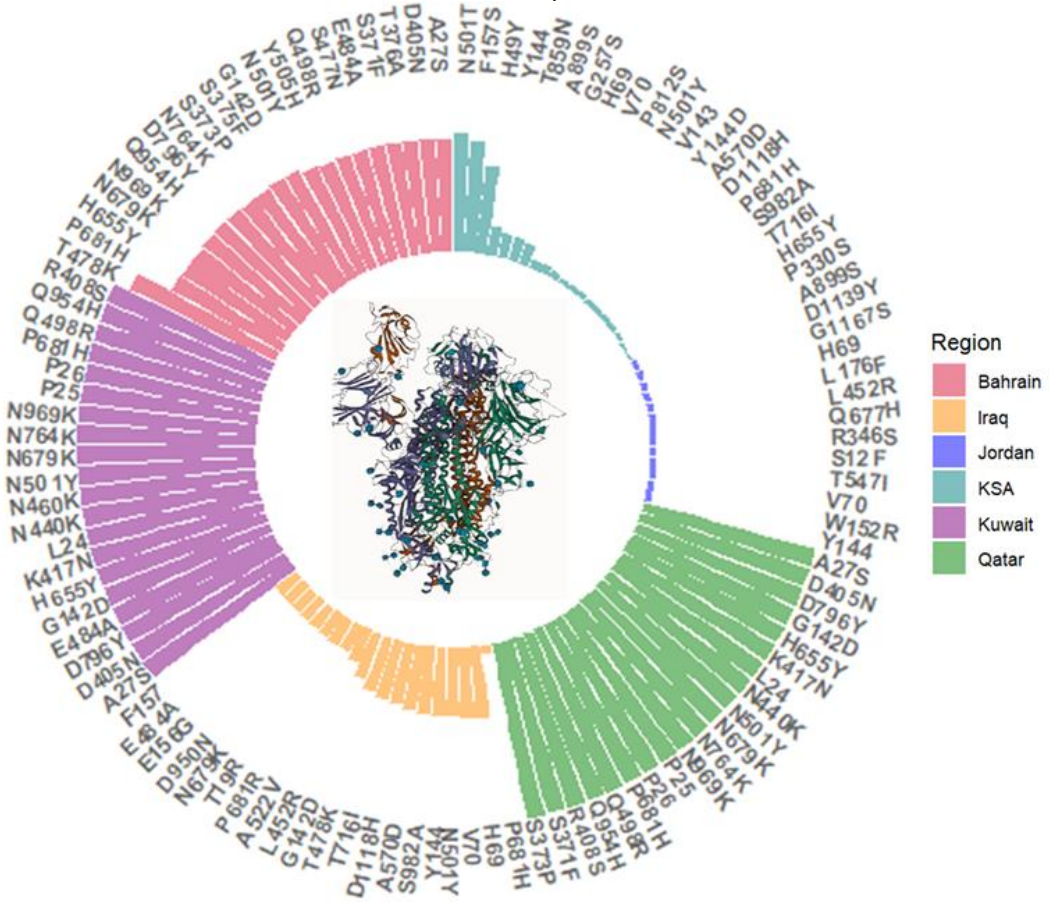

Fig. 2. Depiction of frequencies SARS-CoV-2 spike variants by neighboring regions of KSA. Protein database (PDB) ID = 6BYV, Taxon ID =2697049.

The Spk-covariants (B.1) predominated the KSA, however, the Spk-covariants B.1.1.7, A.28, and B.1.36 demonstrated moderate frequency in the region and the Spk-covariants B.1.351, B.1.456 were the least frequently reported (Figure 3). Moreover, the co-variants CP.1 showed the highest frequency, Spk-BA.2.75.2, BE.3, and BM.1.1.3 exhibited moderate frequency while other co-variants demonstrated equal frequency in in Qatar (Figure 3). The highly frequent Spk-covariant in Kuwait was assessed to be XBB.1 and EG.1 while other covariants were identified with the same frequency in Kuwait (Figure 3). Furthermore, in Jordan, B.1.1 (highly predominant), B.1 (moderately predominant) and other co-variants were equally

predominant in the region (Figure 3). The Spk-covariant B.1.1.7 and B.1.428.1 were highly predominant, while Spk-B.1.617.2, B.1.1, B.1, BA.1.1 moderately frequent, and XBB.1, and XBB.2 were the least frequent covariants in Iraq (Figure 3). Additionally, in Bahrain, Spk-covariant BA.2 predominated the region, while Spk-covariants BA.1 and B.1.1.7 were assessed to be minimally predominant (Fig. 3).

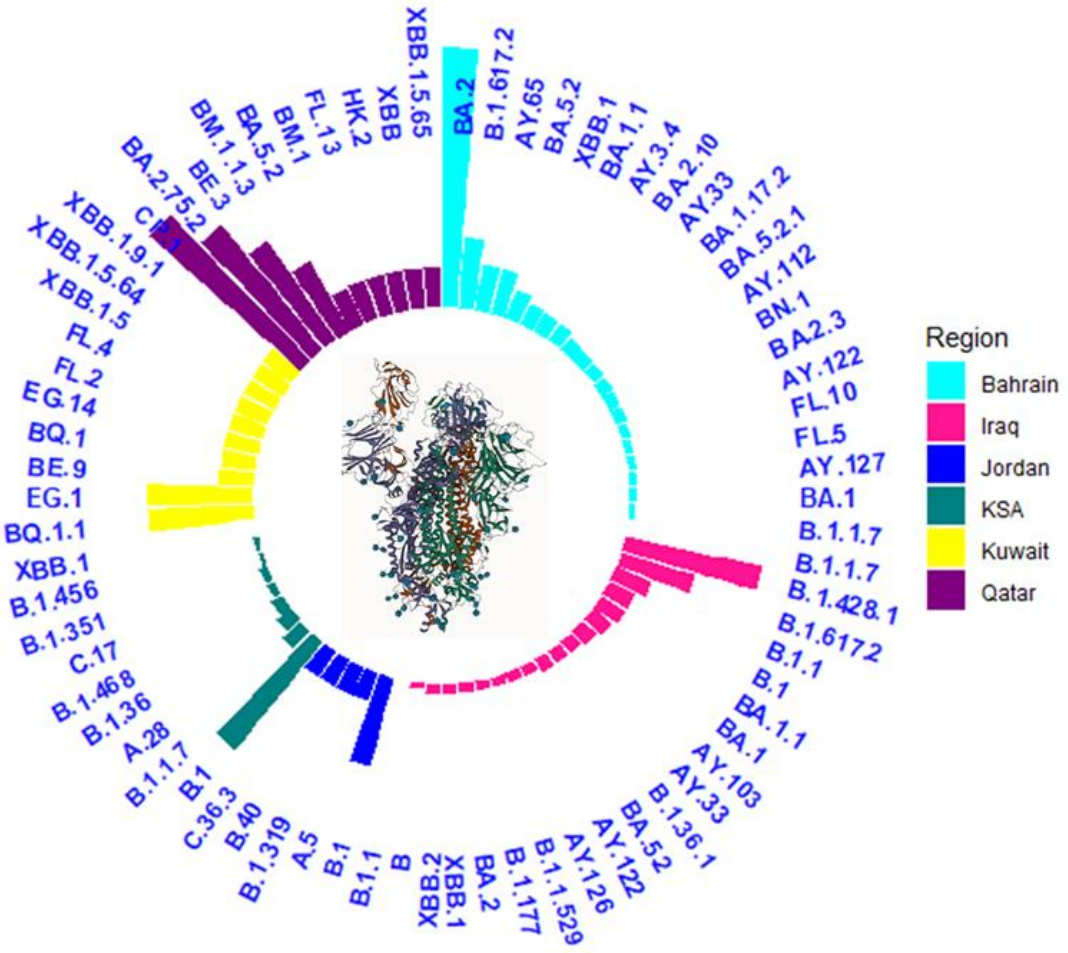

Fig. 3. Depiction of frequencies SARS-CoV-2 spike co-variants by neighboring regions of KSA. Protein database (PDB) ID = 6BYV, Taxon ID =2697049.

4-Cooccurrence and Persistence of the Spk-Variants and Spk-Covariants:

The network analysis demonstrated the clustering of the common variants and covariants among more than one region to understand the occurrences. The Spk-variants N501Y and P681H cooccurred and persisted in KSA and four other neighboring major regions (Qatar, Bahrain, Iraq, and Kuwait) as illustrated in Figure 4. The Spk-variants D405N, G142D, H655Y, and N679K, were observed to co-occur in four different regions (KSA, Bahrain, Kuwait, and KSA) (Fig. 4). Additionally, Spk-A27S, D796Y, E484A, H69 deletion, N969K, Q498R, Q954H, S371F, V70 deletion, and Y144 deletion Spk-variants were characterized in three major regions (Jordan, Iraq, and KSA) while several other variants were identified in one or two regions (Table 1 and Fig. 4).

Moreover, the Spk-covariants (Spk-B.1, B.1.1.7, BA.5.2, and XBB.1) were identified in three different countries (KSA, Jordan, and Iraq) as depicted in Figure 5. AY.122, AY.33, B.1.1, B.1.617.2, BA.1, BA.1.1, and BA2 Spk-covariants were characterized in two major regions, and the rest variants were unique to a particular country (Table 1 and Fig. 5).

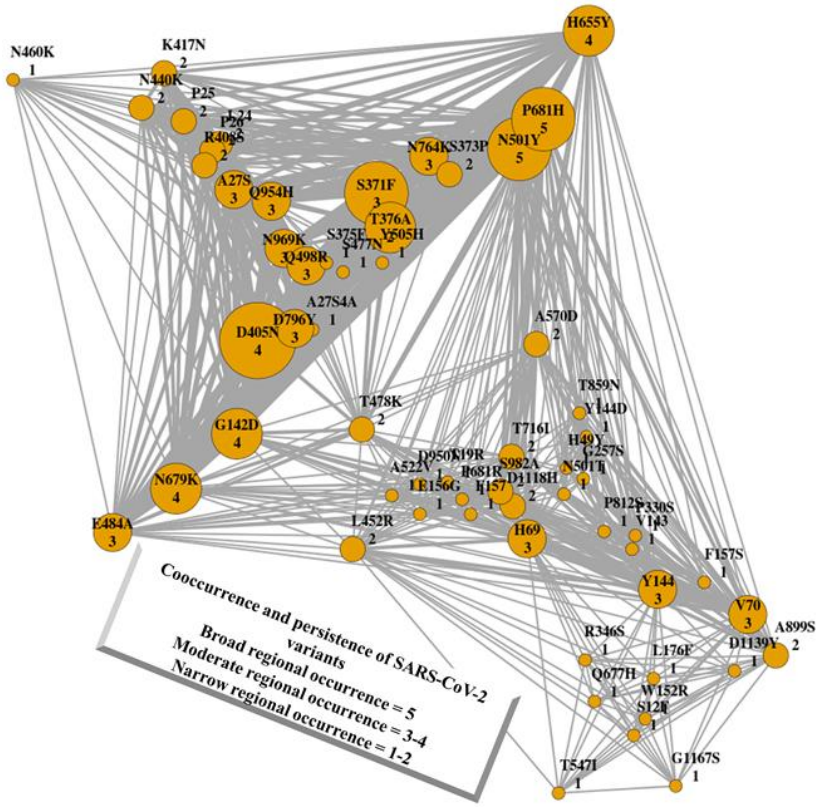

Fig. 4. Cooccurrence and persistence networks for the SARS-CoV-2 spike variants depicting the status of the predominance of the variants in context with geographical region.

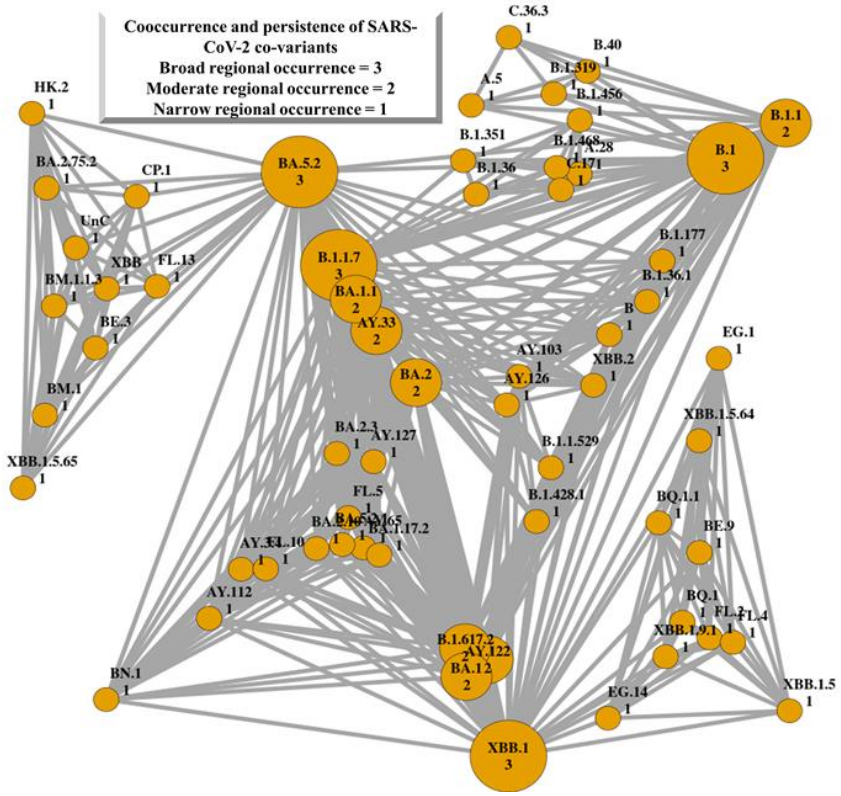

Fig. 5. Cooccurrence and persistence networks for the SARS-CoV-2 spike co-variants depicting the status of the predominance of the variants in context with geographical region.

DISCUSSION

The basis of the continued COVID-19 cases is because of the viral evolution and the emergence of Spk-variants and Spk-covariants (Singh *et al.* 2022). The distribution of these Spk-variants and Spk-covariants/Spk-sub-variants in a geographical region is paramount for shaping control measures, diagnostic intervention, and evaluation of appropriate prophylactic and therapeutic choices in that region as they influence the clinical and biological outcomes of the disease (Mahilkar *et al.* 2022). COVID-19 fresh waves in different regions are being reported even now, therefore, it is imperative to understand patterns of viral evolution (emergence of potential variants and covariants) in a specific geography to curb the future regional outbreak effectively in real-time scenarios.

In the current study, the frequencies of several Spk-variants and Spk-covariants and the magnitude of their cooccurrence in different regions were delineated (Table 1). Spk-variants N501T, F157S, H49Y Y144, T859N, A899S, and G257S were highly prevalent in Saudi Arabia, however, the potential impact of these variants in the patients was not highly associated with an enhancement of the disease severity in the patients (ALGHORIBI *et al.* 2021). The report suggests that the Spk-N501T in combination with Q493K in the S-gene/receptor-binding domain (RBD) has the potential to influence the spike-ACE2 binding-affinity/avidity and interaction (Fiorentini *et al.* 2021) hence it could alter the transmissibility. The F157S, H49Y Y144, T859N, A899S, and G257S Spk-variants have been identified (Ghosh *et al.* 2022) which were later categorized as the VOCs or VoIs based on their potential transmissibility, increased infectivity, and enhanced disease severity in the host (Calistri *et al.* 2021).

The network analysis suggests that the Spk-variant-N501Y and P681H cooccurred and persisted in KSA and four other neighboring major regions highlighting the need for assessment of the prophylactic, diagnostic, and therapeutic countermeasures in context with the impact of these variants. The Spk-N501Y variation together with other seven spike-protein amino acid substitutions constituted the emergence of the UK variant which evolved convergently in P.1 and B.1.351 variants from Brazil and South African region respectively (Komurcu *et al.* 2022; Yang Liu *et al.* 2021). Previous studies suggest that N501Y helps the variant gain consistent fitness for replicating in the epithelial cells, and was identified as the vital determinant of enhanced transmissibility (Y. Liu *et al.* 2021). Additionally, spike-ACE2 binding affinity improved by the N501Y substitution which highlights the concern associated with this adaptive Spk mutation (Kemp *et al.* 2020; Y. Liu *et al.* 2021). Spk-variant-N501Y in Spk-gene-RBD influences the transmissibility and prevalence of this variant (Niu *et al.* 2021). Furthermore, compared to the binding avidity of anti-RBD (IgG) of wild-type to RBD, the RBD of the N501Y-variant showed reduced avidity with the antibody which was reported in a study on the impact of N501Y variation on neutralizing immunoglobulin (Lu *et al.* 2021).

Moreover, another variant P681H which is predominant in most of the neighboring countries of KSA is a significant variation of alfa/Spk-B.1.1.7 VOC. It is considered as a significant substitution which rendered the phenotype more resistant to type I interferons in comparison to the wild-type (M. J. Lista *et al.* 2022). In addition, Spk-B.1.1.7 with Spk-P681H variation increases S1/S2 site-cleavage influencing viral entry and intercellular (cell-to-cell) transmission (Lubinski *et al.* 2021). Furthermore, the significant role of variant P681H in reducing the dependency on endosomal cathepsins to increase viral entry and infectivity has been reported (Maria Jose Lista *et al.* 2022).

Spk-Omicron-variants (Spk-BA.2, BA.4, and BA.5) showed a significant Spk-gene D405N variation (Bugatti *et al.* 2023). The Spk-variant-D405N in combination with other variants such as S371F, R408S, and T376A has been reported to facilitate viral

binding modulation, infectivity alteration, and evasion mechanism (Greaney *et al.* 2021). Another spike-variation (G142D) of the Delta VOC lineages fosters high viral titers in the patients and hosts immune evasion mechanism (L Shen *et al.* 2021). Additionally, the variant G142D has been reported to show its implication in the sequencing methods used for SARS-CoV-2-virus identification for diagnostic purposes (Davis *et al.* 2021).

The SARS-CoV-2-Spk-lineages without Spk-variant-D614G variation were assessed to show the dependence on H655Y variation for enhancing the infectivity and H655Y with Q613H and D614G substitution contributes to stabilizing the spike conformation (Yurkovetskiy *et al.* 2023). Moreover, the substitutions H655Y and D614G exist in Omicron and Gamma variants promoting the fitness of the virion hence contributing to the variant infectivity (Yurkovetskiy *et al.* 2023). The variant N679K with H655Y and P681H was reported to the key Omicron mutations and the variation N679K was found to be reducing the overall level of Spk-protein during the process of Spk-Omicron infection which could have remarkable implications for pathogenesis (Vu *et al.* 2023). The Spk-H69 and V70 deletions in Spk-Alpha-variant/B.1.1.7 have been noticed to decrease the Spk-protein infectivity potential (Meng *et al.* 2021). According to a published report, the variations H69 and V70 delineated in approximately 60 million SARS-CoV-2-virus RNA genomic sequences having their expansion worldwide including KSA (Alvarez-Herrera *et al.* 2024). The Spk-B.1.1.7/501Y.V1 variant having RBD: N501Y variation evolved in the UK's city (Kemp *et al.* 2020) which encompasses eight variations including the two most significant deletions Y144 and (H69/V70) influencing the infectivity, however, they hardly affected the immune evasion (Gupta 2021).

In addition, the CooPer-network analysis result of the study showed that the Spk-covariants-B.1, Spk-B.1.1.7, BA.5.2, and XBB.1 were predominantly found in more than one neighboring region of KSA. Omicron (SARS-CoV-2) harbored maximum Spk-gene variation in comparison to other Spk-covariants: Spk-Alpha-covariant/ B.1. 1.7, Spk-Beta-covariant/B.1.351, Spk-Gamma-covariant/P.1, Spk-Delta-covariant/ B.1.617.2, Spk-Mu/B.1.621, Spk-variant-Epsilon-variants/B.1.427+B.1.429), and Lambda/C.37 (Kandeel *et al.* 2022; Mohseni Afshar *et al.* 2023). These covariants exhibit varying degrees of evasion potential, transmissibility, pathogenicity, and ability to alter disease severity (Mohseni Afshar *et al.* 2023). Spk-covariants-B.1 and B.1.1.7 were the most predominant covariants of the first three COVID-19 waves in certain regions of Asia (Basheer & Zahoor 2021). The covariant B.1.1.7 was a VOC that has 40-50% high transmissibility compared to the wild type, however, it could not escape the neutralizing antibody (X. Shen *et al.* 2021). The clinical manifestation of the Omicron subvariant (BA.5.2)/VOC (Feng *et al.* 2022), and XBB covariants were compared in a study which suggests that the BA.5.2 has higher virulence compared to the XBB contributing to the severe illness and increased death rate (Zhang *et al.* 2023). Other covariants identified in this study are Spk-covariants-AY.122, Spk-covariants-AY.33, B.1.1, B.1.617.2, BA.1, BA.1.1, and BA2 which were found to be confined to one or two neighboring regions raising minimal concern.

The findings of this study underline the necessity to delineate the emerging variants or covariants and their cooccurrence to address issues with the effectiveness of the prophylactic countermeasures (Vo *et al.* 2022). Given the potential implication of the S-gene variation against the prophylactic and therapeutic countermeasures (Xiaoying Shen *et al.* 2021; Wang *et al.* 2021), the scaled-up SARS-CoV-2 sequencing (NGS:next-generation-sequencing), variation-tracking, and characterization of evolving Spk-covariants and Spk-variants could be leveraged for mitigating the vaccine formulation strategies, therapeutic advancement, and development of multivalent vaccines to counter future

outbreaks in KSA and neighboring countries. The outcome of this study also provides the future direction for dealing with outbreaks caused by other human coronaviruses (H-CoVs) such as SARS-CoV and Middle East Respiratory Syndrome coronavirus (MERS-CoV).

Conclusion

A considerable number of spike/RBD-variants and covariants bring about the remarkable modulation in the vital pathophysiology, virulence, and pathogenicity of Spk-variants and Spk-covariants. In the present study, highly frequent variants and covariants of SARS-CoV-2 in KSA and other neighboring countries were delineated. The variants N501T, F157S, and H49Y in KSA, P681H in Iraq, and T478K in Bahrain were the highly frequent S. The Spk-covariants B.1 predominated the KSA, however, the Spk-covariants B.1.1.7, A.28, and B.1.36 demonstrated moderate frequency. Moreover, the co-variants CP.1 showed the highest frequency followed by Spk-covariants-BA.2.75.2, BE.3, and BM.1.1.3 in Qatar. XBB.1 and EG.1 were highly frequent in Kuwait while B. 1 was the most predominant in Jordan. Spk-covariants-B.1.1.7 and B.1.428.1 were predominant in Iraq (Figure 3). BA.1 and B.1.1.7 with high frequency were observed in Bahrain. The Spk-variant-N501Y and P681H cooccurred and persisted in KSA and the majority of the neighboring countries. Moreover, the Spk-covariants-B.1, B.1.1.7, BA.5.2, and Spk-covariants-XBB.1 were observed in three major neighboring countries. Keeping the findings in view, it is noteworthy to bring a surveillance program for tracking variants and covariants with high virulence to minimize potential public health threats, particularly, in immunocompromized and comorbid individuals. Additionally, the research outcome could be leveraged to strategize and formulate policies for countering future SARS-CoV-2 outbreaks in these regions by health authorities effectively.

Declarations:

Ethical Approval: Not applicable

Conflict of Interest: None to declare

Availability of Data And Materials: The data that support the findings of this study are available from the corresponding author (Mohamad I. Rady) upon reasonable request.

Source of Funding: The study did not receive funds.

Acknowledgements: The author acknowledges Mrs. Samia Bashiruddin for her technical assistance (graphical representation).

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