Effective and scalable clustering of SARS-CoV-2 sequences

SARWAN ALI, Georgia State University, Atlanta GA, USA
TAMKANAT-E-ALI, Lahore University of Management Sciences, Lahore, Pakistan
MUHAMMAD ASAD KHAN, Hazara University, Mansehra, Pakistan
IMDADULLAH KHAN, Lahore University of Management Sciences, Lahore, Pakistan
MURRAY PATTERSON, Georgia State University, Atlanta GA, USA

SARS-CoV-2, like any other virus, continues to mutate as it spreads, according to an evolutionary process. Unlike any other virus, the number of currently available sequences of SARS-CoV-2 in public databases such as GISAID is already several million. This amount of data has the potential to uncover the evolutionary dynamics of a virus like never before. However, a million is already several orders of magnitude beyond what can be processed by the traditional methods designed to reconstruct a virus’s evolutionary history, such as those that build a phylogenetic tree. Hence, new and scalable methods will need to be devised in order to make use of the ever increasing number of viral sequences being collected.

Since identifying variants is an important part of understanding the evolution of a virus, in this paper, we propose an approach based on clustering sequences to identify the current major SARS-CoV-2 variants. Using a \( k \)-mer based feature vector generation and efficient feature selection methods, our approach is effective in identifying variants, as well as being efficient and scalable to millions of sequences. Such a clustering method allows us to show the relative proportion of each variant over time, giving the rate of spread of each variant in different locations — something which is important for vaccine development and distribution. We also compute the importance of each amino acid position of the spike protein in identifying a given variant in terms of information gain. Positions of high variant-specific importance tend to agree with those reported by the USA’s Centers for Disease Control and Prevention (CDC), further demonstrating our approach.

CCS Concepts:
• Computing methodologies → Cluster analysis;
• Applied computing → Molecular sequence analysis;
Health informatics.

Additional Key Words and Phrases: SARS-CoV-2, Spike Protein, k-mers, Feature Selection, Clustering, k-means

ACM Reference Format:
Sarwan Ali, Tamkanat-E-Ali, Muhammad Asad Khan, Imdadullah Khan, and Murray Patterson. . Effective and scalable clustering of SARS-CoV-2 sequences. In Proceedings of International Conference on Big Data Research. ACM, New York, NY, USA, 11 pages.

1 INTRODUCTION
The current global COVID-19 pandemic is caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and its variants. SARS-CoV-2 was first detected in Wuhan, China by the end of 2019 (12 December 2019) [44]. Because SARS-CoV-2 is easily transmitted from one person to another, it spread (exponentially) quickly throughout the globe, developing variants such as Alpha and now Delta, which have greatly accelerated this spread. As of July 2021, more than 200 million COVID-19 cases have been reported in 221 countries, with over 4 million deaths [15].
From the perspective of molecular evolution, SARS-CoV-2 continues to diverge as it spreads, developing new variants, like any other virus. Hence, methods for building phylogenetic trees [22] — which have been successful for Ebola, Zika and the Avian Flu — seem apt, because they provide a very complete and detailed evolutionary history of a virus based on molecular sequencing data. The only problem is that such tree building methods can typically process thousands of sequences, while the number of sequences of SARS-CoV-2, available in public databases such as GISAID [21], is already several million. Approaches which first divide the dataset into smaller subsets [17], or use parallelization [31], have allowed the building of trees to scale to tens of thousands of SARS-CoV-2 sequences, however this is still far from closing the gap.

The number of available SARS-CoV-2 sequences forces us to rethink the current methodology used to study the evolutionary dynamics of viruses, and this number will only increase in the coming decades [39]. While building a phylogenetic tree directly on the millions of SARS-CoV-2 sequences seems out of reach, variants of the virus would appear as major clades of the hypothetical SARS-CoV-2 phylogenetic tree. Since the major clades form natural clusters of the sequences, a completely orthogonal approach based on clustering the sequences directly could shed light on this important part of the evolutionary dynamics of SARS-CoV-2 (or viruses in general). Identifying variants has many practical application as well, guiding vaccine design and distribution decisions, as well as informing strategies on how to monitor and prevent future outbreaks. A major advantage of clustering is that it is a widely studied field for which there are techniques which can scale to millions and billions of objects. It remains to decide which (parts of) SARS-CoV-2 sequences to choose, and the best feature vector representation to use for the downstream clustering, which we describe below.

It is common knowledge that the majority of the variation in the SARS-CoV-2 genome takes place in the spike region [28]. Part of this is because the spike region encodes an important function of the virus, namely the spike protein. For this reason, we focus not only on the spike region of the genome, but on the amino acid (protein) sequence encoded by this region (see Figure 1). This allows us to work with a much more compact representation (avoiding the curse of dimensionality). Most of the machine learning (ML) based methods (classification, clustering, etc.) take numerical (real-valued) vectors as an input. Therefore, we cannot work directly with these amino acid sequences if we want to make use of ML based algorithms. Since the order of the amino acids within each sequence also matters, we cannot simply convert the alphabets (amino acids) into numeric characters and apply ML algorithms. A popular approach to preserve the order of the sequences in converting the alphabets vector into numeric vector is to use the one-hot encoding approach [28]. In theory, while the one-hot encoding preserves the order of amino acid sequences, the preserved order is of no use while computing pairwise distance (e.g., euclidean distance) [9]. To deal with this problem, each sequence is first converted into length $k$ substrings (called $k$-mers). These $k$-mers successfully preserve the order of each sequence, that can be crucial to the performance of the classification/clustering tasks [9, 19].

In this paper, we propose a method to quickly and effectively cluster the spike amino acid sequences of SARS-CoV-2. We show that our method performs substantially better than the baseline approach, and successfully clusters the variants into unique clusters with high F1 score. Our contributions in this paper are the following:

![Fig. 1. The SARS-CoV-2 genome is around 29–30kb encoding structural and non-structural proteins. The four structural proteins include spike, envelope, membrane, and nucleocapsid. The spike region comprises 25,384 – 21,563 = 3821 nucleotides, which code for a sequence of 3821 (+ 1 stop codon *) /3 = 1274 amino acids.](image-url)
Effective and scalable clustering of SARS-CoV-2 sequences

International Conference on Big Data Research, 2021

(1) We propose a method based on k-mers for efficient sequence clustering, and show that the quality of clusters as a result of our methods is very high.

(2) We use this to analyze the spread pattern of different variants over time, which can guide vaccination design and distribution strategies.

(3) We compute the importance of each amino acid position in distinguishing a variant, and show that this corresponds to previous studies.

The rest of the paper is organized as follows: Section 2 contains related work. Our proposed approach is detailed in Section 3. A description of the datasets used are given in Section 4. We provide a detailed discussion about the results in Section 5 and then we finally conclude our paper in Section 6.

2 RELATED WORK

Sequence classification and clustering are popular approaches [9, 26], most methods requiring the sequences to be aligned [18]. Such aligned sequences are used to design fixed-length feature vector representations that can be input to classical machine learning (ML) based algorithms. Authors in [14] compute pairwise local and global alignment similarity scores between sequences so that different types of analysis can be performed. Another approach to analyzing the sequences is using heuristic methods. However, these methods require a number of ad-hoc settings (e.g., a penalty for gaps and substitutions). Moreover, these methods may not perform well on specific regions of the genome with high variance. Feature vector based representation has been successfully used in literature for other applications such as missing values prediction in graphs [10], sequence analysis in texts [36–38], biology [19, 27, 29], graph analytics [23, 24], classification of electroencephalography and electromyography sequences [11, 42], detecting security attacks in networks [5], and electricity consumption in smart grids [7, 8]. It is also important to study the conditional dependencies between variables so that their importance can be analysed [4]. Studying the relationships between amino acid positions (attributes) and the class labels (variants) can help us understand which amino acids play a key role in developing specific COVID-19 variants. Information related to mutations and variants could also help the relevant public health departments to study the transmission patterns of different variants, which could help to prevent rapid spread of the underlying virus [1–3, 40].

To address problems that arise in the alignment-based approaches, various alignment-free methods have been proposed [13, 19]. One such method uses k-mers, which preserve the order of the sequences without relying on alignment. Authors in [12] use frequency vectors generated from the k-mers for phylogenetic applications. These methods are successful in constructing accurate phylogenetic trees from several (coding and non-coding) DNA sequences. To measure the (pairwise) similarity between these frequency vectors, different distance measures are used [47]. A recent article [30] proposes a clustering approach for SARS-CoV-2 subtypes identification, however they cluster full-length nucleotide sequences, and use a very different representation and clustering technique.

3 PROPOSED APPROACH

In this section, we first detail the computation of k-mers and the generation of their frequency vectors. We then show how we apply feature selection methods on these frequency vectors, followed by clustering.

3.1 k-mers Computation

The first step of our pipeline is to compute all k-mers of each sequence to map it to a fixed length vector, while allowing its order to be preserved. Given a sequence, the total number of k-mers that can be generated is $N - k + 1$, where $N$ is the total number of elements in the sequence (1274 amino acids in our case), and $k$ is a user-defined parameter for the size of each mer. See Figure 2 for an example. In our experiments we use $k = 3$ — decided using a standard validation set approach [16].
After computing the \( k \)-mers for each sequence, the next step is to generate the numerical representation of these vectors. For this purpose, we design frequency vectors that contain the counts of each \( k \)-mer in the corresponding sequence.

### 3.2 Frequency Vectors Generation

Each sequence \( X \) is over an alphabet \( \Sigma \) (amino acids in our case). Since each frequency vector \( \Phi_k(X) \) must have the same fixed length, this length is \( |\Sigma|^k \), the number of possible \( k \)-mers of \( X \). In our experiments, the length of each frequency vector is 4977.

### 3.3 Feature Selection

After generating the \( k \)-mers based frequency vector \( \Phi_k(X) \) for each sequence \( X \), the next step is to select the most important features in the feature vectors. For this purpose, we use the following two supervised feature selection methods.

#### 3.3.1 Ridge Regression [25]

Ridge Regression (RR) is a popular method for feature selection, which is used to address the collinearity problem that often arises in multiple linear regression [6]. RR works by introducing a bias term to increase the bias and hence improve the variance (i.e., generalization capability of the model). RR changes the slope of the (regression) line and tries to make it more horizontal. RR is beneficial for feature selection because it gives insights on which independent variables are not informative (can reduce the slope close to zero). We can eliminate some of these useless independent variables (hence reduce the dimensions of the data). The objective function of RR is \( \min(\sum \text{of square residuals} + \alpha \times \text{slope}^2) \), where \( \alpha \times \text{slope}^2 \) is a penalty term. After applying RR, we selected 1242 features out of 4977.

#### 3.3.2 Lasso Regression [41]

The Lasso Regression (LR) is another supervised feature selection algorithm similar to RR [33]. The only difference is that it can reduce the slope exactly (i.e., \( \text{slope} = 0 \)) rather than close to zero (as is done by RR). The objective function of LR is \( \min(\sum \text{of square residuals} + \alpha \times |\text{slope}|) \), where the penalty term \( \alpha \times |\text{slope}| \) is now in terms of the cardinality of the slope. This helps to reduce the slope of useless variables exactly to zero. After applying lasso regression, we selected 964 features of 4977.
3.4 Clustering using K-means

After generating $k$-mers, frequency vectors, and feature selection, we use the $K$-means clustering algorithm (with default parameters) to cluster the data. Since we have 5 variants in our data, we used $K = 5$ for in $K$-means (see Section 5.1 for more details regarding the optimal number of clusters).

4 EXPERIMENTAL SETUP

In this section, we first report information related to the dataset. Then we try to find any natural (hidden) clustering in the data by using the t-distributed stochastic neighbor embedding (t-SNE) approach [43]. For the baseline, we use simple $K$-means clustering algorithm without first applying any feature selection on the frequency vectors. To measure the quality of clustering (without and with feature selection methods), we use the weighted F1 score. All experiments are performed on a Core i5 system running the Windows operating system, 32GB memory, and a 2.4 GHz processor. Implementation of the algorithms is done in Python, and the code is available online1.

4.1 Dataset Statistics

We used the (aligned) amino acid sequences corresponding to the spike protein from the largest known database of SARS-CoV-2 sequences, GISAID2. In our dataset, we have 5 most common variants known to date. More information related to the dataset is given in Table 1. After preprocessing (removing missing values and truncated sequences), we end up with 62,657 amino acid sequences.

| Pango Lineage | Region          | Labels | Num. Mutations S-gene / Genome | Num. of sequences |
|---------------|-----------------|--------|-------------------------------|------------------|
| B.1.1.7       | UK [20]         | Alpha  | 8 / 17                        | 13966            |
| B.1.351       | South Africa [20] | Beta  | 9 / 21                        | 1727             |
| B.1.617.2     | India [45]      | Delta  | 8 / 17                        | 7551             |
| P.1           | Brazil [32]     | Gamma  | 10 / 21                       | 26629            |
| B.1.427       | California [46] | Epsilon | 3 / 5                         | 12784            |

Table 1. Variants information and distribution in the dataset. The S/Gen. column represents number of mutations on the S gene / entire genome. Total number of amino acid sequences in our dataset is 62,657.

4.2 Data Visualization

To see if there is any (hidden) clustering in the data, we mapped the data to 2D real vectors using the t-SNE approach. The visualization using t-SNE for the GISAID dataset (before applying Lasso Regression for feature selection) is given in Figure 3 (a). We can see that different variants are scattered everywhere. Although there are small clusters for different variants, we cannot see a clear (complete) cluster for any variant. This analysis shows that any clustering algorithm (like $K$-means) will not work on such type of data directly. We need to perform some preprocessing on the data to cluster the variants effectively.

The visualization using t-SNE for the GISAID dataset (after applying Lasso Regression for feature selection) is given in Figure 3 (b). We can observe that the clusters are more clearly visible after applying feature selection as compared to clustering on frequency vectors without feature selection (as given in Figure 3 (a)). For example, the Epsilon variant (orange color dots) in Figure 3 (a) is scattered everywhere, however, it is concentrated to one

---

1https://github.com/sarwanpasha/Visualization_covid_data
2https://www.gisaid.org/
place (top right side) in Figure 3 (b). The same behavior can be observed for the Beta variant (blue color dots). The Delta variant (green color) is concentrated on bottom left side in Figure 3 (b) forming a single cluster.

Fig. 3. t-SNE plot for the frequency vectors of GISAID dataset along with the variant information.

We also evaluate if there is any (hidden) clustering in the data corresponding to different countries. We took the countries with the most COVID-19 infected patients and show the t-SNE plots in Figure 4. We can observe that there is no clear cluster for any country. Since the mutations in the spike protein take place for different variants, and a country can have patients affected by different variants of the coronavirus, we cannot expect any sort of clustering in this case. Based on these analyses, we evaluate the quality of our clusters using variants information rather than country information.

Fig. 4. t-SNE plot for the frequency vectors of GISAID dataset along with the country information.

The t-SNE plots for feature vectors (with and without feature selection using Lasso Regression) with month information are given in Figure 5. We can again observe that there is no clear clustering for any month in the data (even after the feature selection method). Therefore, since we are unable to find any useful pattern in the data
with respect to month information, we can say that the spread of coronavirus has nothing to do with different seasons over the years (e.g., summer, spring, winter, etc.).

Fig. 5. t-SNE plot for the frequency vectors of GISAID dataset along with the months information.

5 RESULTS AND DISCUSSION
To compute the quality of a clustering, we use the (weighted) F1 score. We label each cluster using the variant which labels the majority of sequences in the cluster (e.g., if the majority of the sequences in a cluster belongs to Alpha variant, we assign the label ‘Alpha’ to that cluster). Now, using these assigned labels, we compute the F1 score for each variant separately. The (weighted) F1 scores for different methods are given in Table 2. We can see that the F1 scores for $K$-means + Ridge are better than simple $K$-means (the baseline), however, they are not better than $K$-means + Lasso for the majority of the variants. One behavior that we can observe for all methods is a low F1 score for the Beta variant. This is due to the much smaller number of sequences available for the Beta variant (see Table 1).

| Methods          | F1 Score (Weighted) for Different Variants |
|------------------|------------------------------------------|
|                  | Alpha | Beta | Delta | Gamma | Epsilon |
| K-means          | 0.3598 | 0.1070 | 0.6110 | 0.6908 | 0.6527 |
| K-means + Ridge  | 0.9992 | 0.0058 | 0.8643 | 0.9998 | 0.7748 |
| K-means + Lasso  | 0.9987 | 0.2705 | 0.9991 | 0.9998 | 0.9704 |

Table 2. Variant-wise F1 (weighted) score for different clustering methods with $k = 5$ for K-means.

The contingency tables of variants versus clusters for $K$-means only, $K$-means + Ridge Regression, and $K$-means + Lasso Regression are given in Table 3. We can see that the variants are not clearly clustered into separate groups if we only use $K$-means without any feature selection method. However, with the feature selection methods (Ridge and Lasso Regression), we can clearly observe that different variants are grouped into their respective clusters. We can also observe that Lasso Regression based feature selection gives us more accurate clusters than Ridge Regression based feature selection. Although Lasso Regression gave us 964 features out of 4977 as compared to 1242 features given by Ridge Regression, those 964 features are a more accurate representation of the original data (as observed in Table 3).
5.1 Optimal value of K for K-means

We use the elbow method to determine the optimal number of clusters (value of $K$ for $K$-means) by fitting the model with values of $K$ ranging from 2 to 14. The quality measure that we use for this analysis is ‘distortion’ that computes the sum of squared distances from each point to its assigned center. Figure 6 shows the distortion score for different values of $K$. To observe the trade-off between the runtime and distortion score, we also plot the (fit/training) runtime (in seconds) in Figure 6 (dashed green line). To determine the optimal value for $K$, we use “knee point detection algorithm (KPDA)” [35]. We can see in Figure 6 that the ideal number of clusters is 4.

Since we know that we have 5 variants in our data, we take $K = 5$ throughout the experiments. It is good, however, to have this independent way of discovering the optimal $K$, and that it is close to the true number. The most likely reason why KPDA selected 4 as the ideal number of clusters is because the Beta variant is not well represented in our data (see Table 1). Moreover, the $K$-means algorithm is not very successful in clustering the Beta variant (as can be seen from its F1 score in Table 2).

![Fig. 6. Sum of squared distances (distortion score) for different numbers of clusters using $K$-means. The blue line shows the distortion score, while the dashed green line shows the runtime (in sec.) for different number of clusters (on the x-axis). The dashed black line shows the optimal number of clusters computed using Elbow method [35].](image)
5.2 The Spread of Variants Over Time

In this section, we analyze the behavior of each variant by considering time as the changing factor. More precisely, we show how the rate of spread of each variant is changing as time passes (from December 2020 to June 2021). Figure 7 (a) shows the relative frequencies of different variants corresponding to different months (for all countries in the data). This study helps us analyze which variants are increasing in number and which ones are waning over time. We can observe that initially, the Delta variant was very rare (in December 2019). However, in the middle of the year 2021 (from April to June), we can see that the Delta variant is spreading very quickly. The behavior of the Epsilon variant is opposite to the Delta variant. We can also observe that the Beta variant is not spreading at an alarming rate, hence there is no threat of the spread of that variant in the near future.

![Variants Spread Over Time](image)

Fig. 7. Month-wise relative frequency of each variant. We observed the relative frequencies of variants for different months separately (from December 2020 to June 2021).

Figure 7 (b) and Figure 7 (c) shows the relative frequency of different variants in USA and India, respectively, from December 2020 to June 2021. We can observe that the Epsilon variant (California variant) was initially present in high numbers in the USA. However, as time passes (and the vaccination process speeds up), we can see the drop in the Epsilon variant. We can also observe that from February 2021 onward, the Gamma variant is spreading at an alarming rate. In the case of India, we can see that, initially, the Alpha variant was the variant of concern. However, after January 2021, Delta becomes the dominantly spreading variant.

5.3 Importance of Each Amino Acid

We compute Information Gain (IG) between each attribute (amino acid position) and the class (variant). The IG is defined as 

\[ IG(Class, position) = H(Class) - H(Class|position) \]

where \( H = \sum_{i \in Class} -p_i \log p_i \) is the entropy, and \( p_i \) is the probability of the class \( i \). The IG values for each attribute are shown in Figure 8 (a higher value is better). The USA’s Centers for Disease Control and Prevention (CDC) declared mutations at certain positions from one variant to the other [34]. We use their mutation information to compare them with the attributes having high IG values in Figure 8. We note that the our high IG value attributes are the same as given by CDC. For example, R452L is present in Epsilon, Delta, and Kappa lineages and sub-lineages. Similarly, K417N, E484K, and N501Y substitutions are present in Beta variant while K417T, E484K, and N501Y substitutions are present in Gamma variant [34] (see Figure 8). The IG values for each attribute are available online for further analysis.\(^3\)

\(^3\)https://github.com/sarwanpasha/Visualization_covid_data/blob/main/correlation_data.csv
6 CONCLUSION

We propose a method to effectively cluster the variants of SARS-CoV-2 using amino acid sequences corresponding to the spike region. Our results show that efficient feature selection methods such as Ridge Regression and Lasso Regression greatly help improve the performance of $K$-means clustering. We also show the rate of spread of each variant with time in different countries. Finally, we show the importance of each amino acid position by computing information gain and compare them with the CDC’s provided information for these same positions. In the future, we will work towards using more SARS-CoV-2 sequences with more variants, as the data continues to accumulate. Using un-supervised feature selection methods would be another interesting future work.

REFERENCES

[1] M. Ahmad, S. Ali, J. Tariq, I. Khan, M. Shabbir, and A. Zaman. 2020. Combinatorial trace method for network immunization. Information Sciences 519 (2020), 215 – 228.

[2] M. Ahmad, J. Tariq, M. Farhan, M. Shabbir, and I. Khan. 2016. Who should receive the vaccine?. In Australasian Data Mining Conference (AusDM). 137–145.

[3] M. Ahmad, J. Tariq, M. Shabbir, and I. Khan. 2017. Spectral Methods for Immunization of Large Networks. Australasian Journal of Information Systems 21 (2017).

[4] S. Ali. 2021. Cache Replacement Algorithm. arXiv preprint arXiv:2107.14646 (2021).

[5] S. Ali, M. Alvi, S. Faizullah, M. Khan, A. Alshanqiti, and I. Khan. 2020. Detecting DDoS Attack on SDN Due to Vulnerabilities in OpenFlow. In International Conference on Advances in the Emerging Computing Technologies (AECT). 1–6.

[6] S. Ali, S. Ciccolella, L. Lucarella, G. D. Vedova, and M. D. Patterson. 2021. Simpler and Faster Development of Tumor Phylogeny Pipelines. bioRxiv 458137 (2021).

[7] S. Ali, H. Mansoor, N. Arshad, and I. Khan. 2019. Short term load forecasting using smart meter data. In International Conference on Future Energy Systems (e-Energy). 419–421.

[8] S. Ali, H. Mansoor, I. Khan, N. Arshad, M. Khan, and S. Faizullah. 2020. Short-Term Load Forecasting Using AMI Data. CoRR abs/1912.12479 (2020).

[9] S. Ali, B. Sahoo, N. Ullah, A. Zelikovskiy, M. D. Patterson, and I. Khan. 2021. A k-mer Based Approach for SARS-CoV-2 Variant Identification. arXiv arXiv:2108.03465 (2021).

[10] S. Ali, M. H. Shakeel, I. Khan, S. Faizullah, and M. A. Khan. 2021. Predicting attributes of nodes using network structure. ACM Transactions on Intelligent Systems and Technology (TIST) 12, 2 (2021), 1–23.

[11] M. Atzori et al. 2014. Electromyography data for non-invasive naturally-controlled robotic hand prostheses. Sci. data 1, 1 (2014), 1–13.

[12] B. Blaisdell. 1996. A measure of the similarity of sets of sequences not requiring sequence alignment. Proceedings of the National Academy of Sciences 83 (1986), 5155–5159.

[13] G. Chang et al. 2014. A novel alignment-free method for genome analysis:HIV-1 subtyping and HEV genotyping. Information Sciences 279 (2014), 776–784.

[14] B. Chowdhury and G. Garai. 2017. A review on multiple sequence alignment from the perspective of genetic algorithm. Genomics 1 (2017), 419–431.
Effective and scalable clustering of SARS-CoV-2 sequences

International Conference on Big Data Research, 2021