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Original Article

# Bioinformatics analysis of rhinovirus capsid proteins VP1-4 sequences for cross-serotype vaccine development

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ABSTRACT

*Background:* Rhinoviruses (RV) are associated with the development and exacerbations of asthma and chronic obstructive pulmonary disease. They've also been linked to more severe diseases like pneumonia, acute bronchiolitis, croup, and otitis media. Because of the hypervariable sequences in the same serotypes, no effective vaccine against rhinoviruses has been developed to date. With the availability of new full-length genome sequences for all RV-A and RV-B serotyped strains, this study used bioinformatics to find a suitable RV strain with the highest similarity matrices to the other strains.
*Methods:* The full genomic sequences of all known different RV-A and -B prototypes were downloaded from the National Centre for Biotechnology Information (NCBI) and divided into minor low-density lipoprotein receptor (LDLR) and major intercellular adhesion molecule groups (ICAM). The sequences were edited using Biological Sequence Alignment Editor, v 7.2.0 (BioEdit software) to study each capsid protein (VP1, VP2, VP3, and VP4) and analyzed using the EMBL-EBI ClustalW server and the more current Clustal Omega tool for the calculation of the identities and similarities.
*Results:* We analyzed and predicted immunogenic motifs from capsid proteins that are conserved across distinct RV serotypes using a bioinformatics technique. The amino acid sequences of VP3 were found to be the most varied, while VP4 was the most conserved protein among all RV-A and RV-B strains. Among all strains studied, RV-74 demonstrated the highest degree of homology to other strains and could be a potential genetic source for recombinant protein production. Nine highly conserved regions with a minimum length of 9-mers were identified, which could serve as potential immune targets against rhinoviruses.
*Conclusion:* Therefore, bioinformatics analysis conducted in the current study has paved the way for the selection of immunogenic targets. Bioinformatically, the ideal strain's capsid protein is suggested to contain the most common RVs immunogenic sites.

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## Introduction

Rhinoviruses (RVs), which were formerly known as human rhinoviruses, were first isolated in 1956 [1]. RVs represent a large number of small non-enveloped viruses of about 28–30 nm in diameter within the genus *Enterovirus* of the *Picornaviridae* family [2]. The viral genome is a positive-sense single-stranded RNA (+ssRNA) of approximately 7200 bases [3]. Within 50 years since

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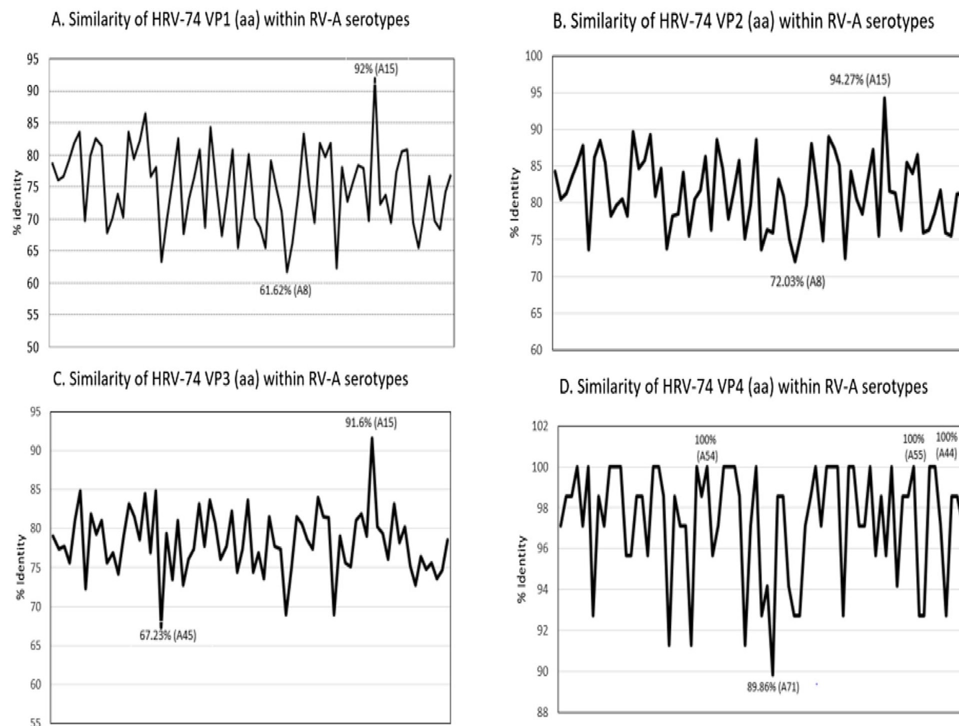


**Fig. 1.** Similarity of HRV-74 VP1 aa with other serotypes within RV-A. HRV-74 VP1 identity within the RV-A group ranged from a low of 61.62% (HRV-8) to a high of 92% (HRV-15) with a median identity of 75%. B. The similarity of HRV-74 VP2 (aa) with other serotypes within RV-A. HRV-74 VP2 identity within the RV-A group ranged from a low of 72.03% (HRV-8) to a high of 94.27% (HRV-15) with a median identity of 81.37%. C. The similarity of HRV-74 VP3 aa with other serotypes within RV-A. HRV-74 VP3 identity within the RV-A group ranged from a low of 67.23% (HRV-45) to a high of 91.6% (HRV-15) with a median identity of 78.24%. D. The similarity of HRV-74 VP4 aa with other serotypes within RV-A. HRV-74 VP4 identity within the RV-A group ranged from a low of 89.86% (HRV-71) to a high of 100% (24 serotype pairs) with a median identity of 97.35%.

their discovery, RVs have been divided into three groups, RV-A,-B, and C, with the latter, RV-C, being reported relatively recently in 2007 [4]. Within each group or species, there are multiple RVs variably designated as "serotypes," "types," or "strains." There are more than 100 types of RV-A and B, while the discovery of new RV-C continues [4–6]. The viral capsid, which surrounds the genomic RNA, is composed of 60 identical copies each of four structural proteins, designated as VP1, VP2, VP3, and VP4. The three more abundant proteins (VP1, VP2, and VP3) are exposed on the capsid surface and account for the virus' antigenic diversity, while the smallest one (VP4) lies at the interface between the capsid and the viral genome. Among the four capsid proteins, VP1 is the largest and the most exposed, and it serves as the site of attachment to the cell surface receptors [7,8]. The surface of the RVs capsid contains neutralization antigenic and host cell-binding sites, which allow the virus to start its replication cycle by attaching to the host cell receptors [9].

Many molecular epidemiological studies of RVs conducted in different regions have revealed that there are no predominant circulating serotypes that could be considered for vaccine development [10,11]. With the high RV burden, which is poorly responsive to the current therapies, alternative approaches to overcome their infections are therefore needed. Eliciting cross-neutralizing antibodies has been considered the ̈holy grail ̈in the search for effective RV vaccines. Capsid proteins (VP1–4) or antigenic peptides corresponding to one of them have been claimed to induce cross-neutralizing antibodies against different RV strains [12,13]. Hence, alternative strategies were applied in an attempt to design a broad-spectrum RV vaccine based on the opposite approach. In the era of genomics, the starting point of designing an ideal vaccine against RVs could be from the available information on their genomes. Recently, the full-length genome sequences of all RV-A and RV-B serotyped strains have been reported [14]. Therefore, this study aimed to use bioinformatics approaches to characterize the sequences of the four capsid proteins (VP1, VP2, VP3, and VP4) of known RV-A and RV-B serotypes to determine the phylogenetic relationship between strains and to reveal the ideal strain that possesses genome sequences that are highly identical to other strains and ultimately to identify the highly conserved antigenic regions across the majority of RVs A and B within the capsid proteins to serve as vaccine candidates in future studies.

## Materials and methods

### *Data collection*

The complete genome sequences of all known distinct RV-A and-B prototypes downloaded from the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) except HRV-87 as it had been reclassified as a human enterovirus [15]. VP1 data under GenBank accession number AY355180 to AY355281, as described [16], was adapted to confirm the accuracy of the analysis.

### *Characterization of rhinovirus (RV) capsid protein VP1, VP2, VP3, and VP4*

The obtained RV sequences (RV-A, n = 75; RV-B, n = 25) were grouped based on their original classification. The RV-A group was divided into two sub-groups: minor low-density lipoprotein receptor (LDLR) (n = 12) and major intercellular adhesion molecule (ICAM) (n = 63). Upon grouping, the sequences were edited using Biological Sequence Alignment Editor, v 7.2.0 (BioEdit software) to study each capsid protein (VP1, VP2, VP3, and VP4) independently [17]. Sequence alignments were performed for all the available

complete capsid proteins from both HRV-A and B by using the MUSCLE multiple sequence alignment algorithms which offers a range of options that provide improved speed and/or alignment accuracy compared with currently available programs and is the fastest of the tested methods for large numbers of sequences [18]. The conserved regions that are common across all RV-A and B serotypes, exhibiting at least 80% representation, were selected. Similarly, selected sequences were further analyzed using the EMBL-EBI ClustalW server and the more current Clustal Omega tool (The European Bioinformatics Institute; http://www.ebi.ac.uk) for the calculation of the identities and similarities for the selected sequences. Subsequently, consensus sequences and distance matrices were generated for each VP protein.

*Entropy calculation*

Mining for conserved sequences among the aligned sequences was performed by determining the entropy of regions with at least nine amino acids in the length and maximum average entropy of 0.2. The maximum entropy per position was 0.2, with gaps limited to 2 per segment. Contiguous gaps were limited to 1 in any segment. This minimum length of amino acids was chosen because it represents the binding core length of a majority of human leukocyte antigen (HLA) restricted T-cell determinants [19,20].

*B-cell epitope prediction*

There are several bioinformatics tools for assessing and predicting B cell epitopes [14,21]. We chose to determine the continuous B cell epitopes as it is mainly based on the properties of the amino acids in the peptide, such as the hydrophilicity, charge, exposed surface area, and secondary structure. For discontinuous epitope prediction, a pre-requisite is the 3-D structure of the antigen. We used the ABCPred software (www.imtech.res.in/raghava/abcpred/) for continuous B-cell epitope prediction. Briefly, the amino acid sequence of the VP candidate genes was uploaded to the ABCPred server to be tested against a preselected dataset of 700 unique experimentally proven B-cell epitopes, which is a subset of the BCIPEP database, as well as 700 non-epitopes or random peptides. The default threshold was chosen for the analysis.

## Results

Phylogenetic analysis of the retrieved sequences of the RV capsid proteins was performed for the future goal of constructing recombinant proteins for use as vaccine candidates. This facilitated the determination of the relationships between strains and identification of the ïdealštrain among strains that are highly identical to other strains. Also, the prior knowledge of the variability among prototype strains has provided a unique opportunity to enhance the optimal selection of the immunogens, and afterward, the selection of strains examined for cross-reactivity. Alignment analysis showed that full-length VP1, VP2, and VP3 amino acid (aa) sequences were found to be variable between strains and varied in length, with VP4 as the exception. VP4 possessed the shortest sequence with only 69 aa residues in length and was found to be the most conserved protein among all capsid proteins.

*Characterization of VP1 sequence*

The pair-wise analysis revealed that VP1 amino acid sequences within individual groups were not well conserved with an average identity of 70.61% for RV-A, which ranged from 57.24% (HRV-45 and HRV-49) to 98.59% (HRV-8 and -95). On the other hand, the average identity of VP1 aa within RV-B was 76.27%, with a range of 66.9% (HRV-14 and -84) to 93.24% (HRV-70 and -91). There were eighteen and seven pairs of serotypes with a similarity of 90% or more for RV-A and RV-B, respectively. Among the RV-A strains analyzed (n = 75), the VP1 amino acid sequence of HRV-74 had the highest degree of median identity to the other strains (75%), while the lowest median identity was demonstrated by HRV-45 (62.09%). Besides, a consensus sequence (305 aa) that represented the 75 aligned VP1 aa sequences was obtained from multiple sequence alignment. Further analysis shows HRV-74 has the highest homology to this consensus sequence (86%) Based on these findings, HRV-74 has been shortlisted as the template source for the bioinformatics design of recombinant proteins to be used in future vaccine development studies. Hence, as shown in Fig. 1, when we used HRV-74 as the baseline for comparison to the VP1 sequences from other RV-A strains, identity within the RV-A group ranged from a low of 61.62% (HRV-8) to a high of 92% (HRV-15). Further, Fig. 2 help in understanding the sequence of alignment of the HRV74 VP1 aa sequence against the consensus VP1 sequence.

Fig. 2 depicts the sequence alignment of the HRV74 VP1 aa sequence against the consensus VP1 sequence.

*Characterization of VP2 sequences*

The level of VP2 aa identity withinRV-A ranged from 70.11% (HRV-23 and -8) to 99.23% (HRV-8 and 95) with a median identity of 80.27%. Meanwhile, RV-B VP2 amino acid sequence identity ranged from 73.95% (HRV-79 and 84) to 96.18% (HRV-70 and HRV-17) with a median identity of 81.28%. There were eighty and twenty-four serotype pairs that have an amino acid sequence identity of more than 90% for RV-A and -B, respectively. Also, a consensus sequence (270 aa) that represented the 75 aligned VP2 aa sequences was obtained. The consensus identity within the RV-A group ranged from 74.7% (HRV-8) to 91.92% (HRV-21), with a median identity of 84.7%. Our analysis also showed that 130 residues (48.14% of the VP2 full length) at different positions were 100% conserved across all RV-A. Fig. 3 shows the sequence alignment of HRV74 VP2 aa to the consensus sequence.

*Characterization of VP3 sequences*

Within RV-A, the level of VP3 aa identity ranged from 63.14% (HRV-30 and 8) to 100% (HRV-8 and 95) with a median identity of 76.11%. On the other hand, the RV-B amino acid sequence identity of the respective protein ranged from 71.61% (HRV-3 and 27) to 97.88% (HRV-70 and HRV-17) with the median identity of 81.38%. There were sixty-one, and twenty-seven serotype pairs that have an amino acid sequence identity of more than 90% for RV-A and -B, respectively. Besides, the cconsensus sequence (241 aa) that represented the 75 aligned VP3 aa sequences of RV-A serotypes was generated. The consensus identity within the RV-A group ranged from 72.27% (HRV-8 and 95) to 88.24% (HRV-43) with a median identity of 80.38%. Our analysis also showed that 90 residues (37.3% of the VP3 full length) at different positions are 100% conserved across all RV-A. The sequence alignment of HRV74 VP3 aa to that of the consensus sequence is shown in Fig. 4.

*Characterization of VP4 sequences*

Lastly, within RV-A, the level of VP4 aa identity ranged from 88.41% (several serotypes) to 100% (332 serotypes pairs) with a median identity of 95.91%. Meanwhile, RV-B amino acid sequence identity ranged from 88.41% (HRV-26 and 48) to 100% (5 serotype pairs) with a median identity of 95.3%,oted that VP4 is the only capsid protein whose median identity of serotype pairs within-group ais more than group B. In contrast to other capsid proteins, only seventy-six and seven serotype pairs have an amino acid sequence identity less than 90% for RV-A and B, respectively. Besides, the

```
CLUSTAL O (1.2.4) multiple sequence alignments

VP1-cons    NPVENYVDEVLNEVLVVPNIKESHPTTSNSAPALDAAETGHTSNVQPEDHIETRYVQTSQ   60
[A74]       NPVENYIDEVLNEVLVVPNIRESHSSTSNSAPALDAAETGHTSNVQPEDNVETRYVQTSQ   60
            ******:*************:*** :***********************::*********

VP1-cons    TRDEMSIESFLGHSGCIHISTLEVDSDYDMYNDVYNAEGEHFTKWKINLQEMAQIRRKFE   120
[A74]       TRDEMSVESFLGRSGCIHISHLKIDY------TNYNVEGKNFTKWQINLKEMAQIRRKFE   114
            ******:*****:******* *.:*       ** **::****:***:**********

VP1-cons    LFTYVRFDSEITLVPCIAAKGDDIGHVVMQYMYVPPGAPIPKKRDDYTWQSGTNASVFWQ   180
[A74]       LFTYVRFDSEVTLVPCIAAKSDNIGHVVMQYMYVPPGAPLPKKRDDYTWQSGTNASVFWQ   174
            **********:********* *:****************:********************

VP1-cons    HGQPYPRFSLPFLSIASAYYMFYDGYDGDSPGSRYGTVVTNDMGTLCSRIVTEEKQKHKV   240
[A74]       HGQPYPRFSLPFLSIASAYYMFYDGYDGDSTESHYGTVVTNDMGTLCSRIVTEEH-DARV   233
            ****************************** *:*********************: .:*

VP1-cons    EITTRIYHKAKHVKAWCPRPPRAVEPYTHTHVTNYKPQDGEPTVEVTTAIKPRANIKTVT   300
[A74]       EITTRVYHKAKHVKAWCPRPPRAVE-YTHTHVTNYKPQEGDVTTVIPT----RRSIVNV-   287
            *****:******************* ************:*: *.:*    *.*.*

VP1-cons    AVEN   304
[A74]       ----   287
```

**Fig. 2.** Alignment of the RV-A VP1 consensus sequence and HRV-74 VP1 sequence. The consensus sequence was built based on the 75 full-length RV-A VP1 aa sequences. An *(asterisk) indicates positions that have a single, fully conserved residue. A: (colon) indicates conservation between groups of strongly similar properties. A. (dot) indicates conservation between groups of weakly similar properties.

```
CLUSTAL 2.1 multiple sequence alignment


VP2-A-cons      SPTVEACGYSDRIIQITRGDSTITSQDVANAVVGYGVWPHYLTPQDATAIDKPTQPDTSS 60
[A74]           SPTVEACGYSDRIIQITRGDSTITSQDVANAVVGYGVWPHYLTPEDATAIDKPSHPDTSA 60
                ********************************************:*********::****:

VP2-A-cons      NRFYTLESKTWTSTSKGWWWKLPDALKDMGIFGENMFYHFLGRSGYTVHVQCNASKFHQG 120
[A74]           NRFYTLESKLWNGESKGWWWKLPDALRDMGVFGQNMYYHFLGRNGYTVHVQCNSSKFHQG 120
                ********* *.. *************:***:**:**:*******.**********:******

VP2-A-cons      TLIVAMIPEHQLASATTGNVTAGYNYTHPGEAGRDVGQTRRENNLDKQPSDDNWLNFDGT 180
[A74]           TLIVAMIPEHQLASAERGNVTAGYKLTHPGEAGRDVSITTREHTS-RQPSDDNWLNFDGT 179
                ****************  ********: ***********. * **:.   :**************

VP2-A-cons      LLGNITIFPHQFINLRSNNSATIIVPYVNAVPMDSMLRHNNWSLVIIPICPLESDGGATN 240
[A74]           LLGNITIFPHQFINLRSNNSATLIVPYVNAVPMDSMLRHNNWSLVIIPICPLEVSGNEVN 239
                ************************:**********************************  .*. .*

VP2-A-cons      TVPITISISPMCAEFSGARARARSQAAAQQ 270
[A74]           TVPITISISPMFAEFSGARARSQ------- 262
                ************ **********::
```

**Fig. 3.** Alignment of consensus of the RV-A VP2 sequence and HRV-74 VP2 sequence. The alignment shows a homology of 87.2% between both sequences. The consensus was built based on the 75 full-length RV-A VP2 aa sequences. An * (asterisk) indicates positions that have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties. A. (dot) indicates conservation between groups of weakly similar properties.

```
CLUSTAL 2.1 multiple sequence alignment


[A74]           GLPVMMTPGSGQFMTTDDFQSPSALPWFHPTKEISIPGQVRNLVEMCQVDTLIPINNTAG 60
VP3-A-Cons      GLPVMLTPGSGQFMTTDDFQSPSALPWFHPTKEIFIPGEVKNLIEMCQVDTLIPINNTEA 60
                *****:****************************  ***:*:**:***************  .

[A74]           N-VNNVNMYTVLLERKPD--LMQEVFAIKVDIASQPLATTMIGEISSYFTHWTGSLRFSF 117
VP3-A-Cons      N-IRNVNMYTVQLTRQTD-DLAQEIFAIKVDIASQPLATTLIGEIASYYTHWTGSLRFSF 118
                *  :.******* * *:.*  * **:****************:****:**:***********

[A74]           MFCGTANSTLKLLLAYTPPGIDKPRSRKDAMLGTHVVWDVGLQSTISMIVPWVSASHFRN 177
VP3-A-Cons      MFCGTANTTLKLLLAYTPPGIAKPTSRKEAMLGTHVVWDVGLQSTISMVVPWVSASHFRN 178
                *******:*************** ** ***:*******************:***********

[A74]           TTPDTFSSAGFITCWYQTNLVVPPNTPVTARMLCFVSGCKDFCLRMARDTDLHKQRGNIT 237
VP3-A-Cons      TTPDTYSLAGYITCWYQTNLVVPPNTPNTADMLCFVSGCKDFCLRMARDTNLHKQSGPIT 238
                *****:* **:***************** ** ******************:**** * **

[A74]           Q 238
VP3-A-Cons      Q 239
                *
```

**Fig. 4.** Alignment of consensus of the RV-A VP3 sequence and HRV-74 VP3 sequence. The alignment shows homology of 87% between both sequences. The consensus was built based on the 75 full-length RV-A VP3 aa sequences. An * (asterisk) indicates positions that have a single, fully conserved residue. A: (colon) indicates conservation between groups of strongly similar properties. A. (dot) indicates conservation between groups of weakly similar properties.

```
CLUSTAL 2.1 multiple sequence alignment


VP4-A-Cons      MGAQVSRQNVGTHSTQNSVSNGSSLNYFNINYFKDAASSGASKLEFSQDPSKFTDPVKDV 60
VP4-A74         MGAQVSRQNVGTHSTQNSVSNGSSLNYFNINYFKDAASSGASKLEFSQDPSKFTDPVKDV 60
                ************************************************************

VP4-A-Cons      LEKGIPTLQ 69
VP4-A74         LEKGIPTLQ 69
                *********
```

**Fig. 5.** Alignment of consensus of the RV-A VP4 sequence and HRV-74 VP4 sequence. The alignment shows a homology of 100% between both sequences. The consensus was built based on the 75 full-length RV-A VP4 aa sequences. An * (asterisk) indicates positions that have a single, fully conserved residue.

consensus sequence (69 aa) that represented the 75 aligned VP4 aa sequences of RV-A serotypes was generated. The consensus identity within the RV-A group ranged from 89.86% (HRV-71) to 100% (25 serotype pairs) with a median identity of 97.37%. Fig. 5 depicts the sequence alignment between the consensus sequence and HRV74 aa sequence of VP4. Further analysis also showed that 55 residues (79.7% of the VP4 full length) at different positions are 100% conserved across all RV-A.

*Identification of potential rhinovirus VP1, VP2, VP3, and VP4 conserved regions*

In this study, upon multiple alignments of VP1 aa sequences, several conserved regions across each selected group with a minimum length of 9 residues and maximum entropy 0.2 were found. Our analysis showed that there were three conserved regions in the RV-A major receptor group, while six motifs were detected among the minor receptor group (Table 1). Upon multiple sequence alignment of all RV-A, we identified three highly conserved regions for the VP1 I-CAM1 region (Underlined in Table 1). Meanwhile, our analysis of RV-B showed no conservative motifs using the applied criteria as described. However, several conserved motifs within the group were observed once the maximum entropy was changed to 0.5, as shown in Table 1. Also, several residues appeared to be conserved across all studied RV prototype strains (RV-A & -B).

Our analysis of the VP2 aa sequence indicated that the VP2 aa sequence was more conserved and showed less variability in both groups as compared with VP1 protein. Several conserved regions across each selected group with a minimum length of 9 residues and maximum entropy of 0.2 were found. The analysis of the RV-A major receptor group showed three conserved regions, while seven motifs were detected among the minor receptor group (Table 2). Unlike VP1, VP2 of RV-B shows six conserved motifs using the applied criteria, as shown in Table 2. Also, several residues within the detected regions appear to be conserved across all studied RV (RV-A & -B) prototype strains (Table 2).

Our findings revealed that the VP3 aa sequences were the most variable among the VP1–4 capsid proteins. Unlike other capsid proteins, no conserved region in group A with a minimum length of 9 residues and maximum entropy 0.2 was found. However, once we modified the selection to 9 residues and maximum average entropy of 0.2 with disabling of the maximum entropy per position, five not so well-conserved motifs were observed within RV-A ICAM-1 group (Table 3). On the other hand, the analysis of the minor receptor (LDLR) group protein shows five highly conserved regions, with only two detected among RV-B for the respective protein (Table 3). Besides, there were fewer residues within the detected regions that appear to be conserved across all studied RV (RV-A & -B) prototype strains (highlighted in Table 3).

In contrast with VP3, our results showed that the VP4 aa sequence was the most conserved compared to the other four VPs. Our analysis showed that 72% of the VP4 sequence is highly conserved within the HRV-A primary receptor. Furthermore, VP4 of the minor receptor (n = 10) contained three highly conserved segments, which account for 85% of its total length. HRV-B VP4, in contrast, contains less conserved regions, which exhibited only 25% of the protein's total length. Upon multiple sequence alignment of all HRV-A, three highly conserved regions were identified for VP4 (Underlined in Table 4).

*Prediction of continuous B-cell linear epitopes*

The B-cells epitopes prediction was performed using ABCPred Prediction Software among the conserved regions identified within the VP1, VP2, VP3, and VP4 protein sequences, as shown in Table 5. Among these, VP4 was found to have the ideal epitope regions followed by VP2. VP1 and VP3 did not have any regions that satisfied the minimum threshold of the epitope prediction software. Our findings indicate that the conserved regions identified within the VP4 region of the protein sequences are potential immune targets against RVs.

The predicted B cell epitopes are ranked according to their score obtained by the trained recurrent neural network. A higher score of the peptide means a higher probability of being as an epitope. All the peptides shown with * are above the threshold value chosen.

## Discussion

In this study, we adopted bioinformatics analysis on available RV prototype strains intending to characterize and understand the relationship between strains, to identify a potent strain that is highly and ideally similar to other strains, and to analyze all the immunogenic capsid proteins of all strains to identify the highly conserved amino acid sequences. Through aa sequence analysis, the four capsid proteins (VP1−4) were found to be variable in length and sequence among different strains. Our results revealed that VP3 is the most variable protein, followed by VP1 and VP2 proteins, while VP4 is the most conserved protein. Our findings in this study are in agreement with previous studies [23,24].

Our findings demonstrated that within the group, the amino acid sequences of VP4 have the most significant degree of identity, ranging from as low as 88.41% (several serotype pairs) to as high as 100% (337 serotype pairs). Even though VP4 is located inside the virion, the bioinformatics findings strongly suggest that VP4 may be considered an immunogenic particle. A previous study [26] showed that antibodies against the N-terminus of VP4 HRV14 are capable of neutralizing viral infectivity. Another study [25], also demonstrated that the HRV14 capsid is dynamic and transiently displays the buried N-termini of viral VP1 and VP4 by breathing phenomenon. Meanwhile, a recent study also indicated that VP0 (VP4 + VP2) induces cross-reactive immune responses. Hence, it is strongly suggested that VP4 could be a candidate for the RV vaccine [27]. Meanwhile, VP1−3 proteins are known to be located on the surface of the RV capsids [28]. Hence, these VP1−3 capsid proteins are expected to be amenable to vaccine development. However, our findings revealed that they are highly variable antigenic proteins, which could hinder their potential as vaccine candidates that could elicit cross-neutralizing antibodies in our case. Furthermore, there is limited evidence for their implications in vaccination. Previous studies reported that VP1 protein had been considered as having the highest potential as a vaccine candidate [8,29] because it is the most surface-exposed and immunodominant of all the four capsid proteins.

The cryoelectron microscopy of rhinovirus 14 virions contains the octanucleotide high-density segment of the RNA genome that binds with the VP-2 subunits. It has been shown that the binding of ICAM-1 to rhinovirus 14 is needed to prepare the virus for genome discharge at acidic pH. Arrangement of the rhinovirus 14–ICAM-1 complex incites conformational changes to the rhinovirus 14 cap-

**Table 1**
Conserved sequences identified within RV-VP1 capsid proteins.

| Sp. | Receptor | No. | Peptide length | Region** | Consensus |
|---|---|---|---|---|---|
| RV-A | ICAM-1 | 1 | 9 | 11 to 19 | LNEVLVVPN |
| | | 2 | 9 | 34 to 42 | **L**D**A**A**ETG**H**T** |
| | | 3 | 11 | 197 to 207 | **ASAY**YM**FYDGY** |
| | LDLR | 1 | 11 | 9 to 20 | EVLNEVLVVPNI |
| | | 2 | 16 | 34 to 49 | LDAAETGHTSNVQPED |
| | | 3 | 9 | 68 to 76 | ESFLGRSGC |
| | | 4 | 10 | 103 to 112 | LQEMAQIRRK |
| | | 5 | 11 | 143 to 153 | MQYMYVPPGAP |
| | | 6 | 11 | 190 to 200 | ASAYYMFYDGY |
| RV-B | ICAM-1 | 1 | 9 | 70 to 78 | FLGRAACVH<br>****:**** |
| | | 2 | 10 | 114 to 123 | LSSLVQLRKK<br>:*****:*:* |
| | | 3 | 15 | 127 to 141 | FTYVRFDSEYTILAT<br>***.*********:** |
| | | 4 | 14 | 173 to 186 | DYTWQSASNPSVFF<br>*:*****:****** |
| | | 5 | 14 | 202 to 215 | ASAYNCFYDGYSHD<br>****..********* |
| | | 6 | 10 | 221 to 230 | YGINVLNHMG<br>***:****** |

Sp.: Species. No.: Peptide No. detected in each sub-group. (**): Numbering of amino acids is based on consensus sequence generated for the studied protein. The underlined sequence is the consensus of highly conserved motifs across all RV-A (n = 75). Highlighted residues are conserved across all studied RV-A & -B (n = 100). An * (asterisk) indicates positions that have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties. A. (dot) indicates conservation between groups of weakly similar properties.

**Table 2**
Conserved sequences identified within RV-VP2 capsid proteins.

| Species | Receptor | No. | Segment length | Region** | Consensus |
|---|---|---|---|---|---|
| RV-A | VP2-ICAM | 1 | 9 | 4 to 12 | VEACGYSDR |
| | | 2 | 10 | 77 to 86 | GWWWKLPDAL |
| | | 3 | 11 | 207 to 217 | PYVNAVPMDSM |
| | VP2-LDLR | 1 | 10 | 4 to 13 | VEACGYSDRI |
| | | 2 | 17 | 15 to 31 | QITRGDSTITSQDVANA |
| | | 3 | 11 | 56 to 66 | PDTSSNRFYTL |
| | | 4 | 12 | 75 to 86 | SKGWWWKLPDAL |
| | | 5 | 11 | 173 to 183 | WLNFDGTLLGN |
| | | 6 | 14 | 188 to 201 | PHQFINLRSNNSAT |
| | | 7 | 11 | 205 to 215 | PYVNAVPMDSM |
| RV-B | VP2 -ICAM | 1 | 9 | 5 to 13 | EACGYSDRV |
| | | 2 | 12 | 19 to 30 | GNSTITTQEAAN |
| | | 3 | 13 | 75 to 87 | SKGWCWKLPDALK |
| | | 4 | 12 | 109 to 120 | HVQCNATKFHSG |
| | | 5 | 16 | 183 to 198 | IFPHQFINLRTNNTAT |
| | | 6 | 13 | 202 to 214 | PYINSVPMDSMTRHNN<br>**:**.*:********** |

(**): Numbering of amino acids is based on the consensus sequence generated for the studied protein. The underlined sequence is the consensus of fully conserved motifs across all RV—A (n = 75). Highlighted residues are conserved across all studied RV—A & -B (n = 100). An * (asterisk) indicates positions that have a single, fully conserved residue. A: (colon) indicates conservation between groups of strongly similar properties. A. (dot) indicates conservation between groups of weakly similar properties.

sid, including the movement of the C ends of VP4 subunits, which become ready for discharge through pores that open in the capsids of initiated particles. [30]. However, further details for VP1 and others remain elusive even though several studies have been conducted for decades [31]. VP2 is highly suggested to be considered in the vaccination approach. In particular, VP2 has been considered in several studies as a target for RV vaccine development, anti-peptides derived from VP2 showed neutralizing activity and have been suggested to play a role in the future of RV vaccination [32–34]. VP3 is quite different from VP2. First, it is shorter (236–241 aa) but is more variable in contrast to VP4, which is shorter (69 aa) but more conserved. The median identity of VP3 within group A is 76.1%, while it is 80.3% for B. The analysis carried out shows that there are 90 residues (37.3% of the full length) that are fully conserved across all RV-A. In terms of numbers, VP3 is more conserved than VP1, but no well-conserved motifs with nine residues or more were identified. This feature has allowed its categorization (VP3) as the most variable protein among the four studied proteins. Just like the bioinformatics analysis, limited studies explain the role of VP3 in immunogenicity. Very recently, those who investigated the specificities of antibody responses in individuals (with and without asthma after experimental HRV-16 infection) found no relevant antibody responses specific for either VP3 or VP4. Another study investigated the most highly immunogenic neutralization antigens using neutralizing monoclonal antibodies raised against native virion [35,36]. This is one of the early pieces of evidence that shows VP1-VP3 playing a role in RVs immunogenicity. To date, NimIA-NimIII has been displayed as raised features in all resolved structures of RV-A and -B strains. In contrast to VP1, the physical loops of HRV-C15 for putative NimII and NimIII analogs were found to be similar to HRV-A16 and HRV-B14 [37]. In contrast to the three surface-exposed proteins, only seventy-six (2.7%) and seven (2.3%) serotype pairs had an amino acid sequence identity of less than 90% for VP4 of RV-A and B, respectively. Interestingly, the average of median identities in the respective groups was 95.91% (90.95%–97.35%) versus 95.3%. It is the only capsid protein whose

**Table 3**
Conserved sequences identified within RV-VP3 capsid proteins.

| Species Group | Receptor | No. | Segment length | Region** | Consensus |
|---|---|---|---|---|---|
| RV-A | VP3-ICAM | 1 | 12 | 7 to 18 | TPGSGQFMTTDD<br>******:**** |
| | | 2 | 18 | 112 to 119 | THWTGSLRFSFMFCGTA<br>*:****:*..::***:* |
| | | 3 | 11 | 158 to 168 | WDVGLQSTISM<br>**:******: |
| | | 4 | 9 | 171 to 179 | PWVSASHFR<br>**:*:::* |
| | | 5 | 15 | 219 to 233 | CKDFCLRMARDTNLH<br>*:.****:****.* |
| | VP3-LDLR | 1 | 12 | 7 to 18 | TPGSGQFMTTDD |
| | | 2 | 11 | 24 to 34 | ALPWYHPTKEI |
| | | 3 | 14 | 107 to 120 | THWTGSLRFSFMFC |
| | | 4 | 11 | 128 to 138 | KLLLAYTPPGI |
| | | 5 | 12 | 216 to 227 | CKDFCLRMARDT |
| RV-B | VP3-ICAM | 1 | 13 | 12 to 24 | QFLTTDDRQSPSA |
| | | 2 | 12 | 116 to 127 | MYTGPALSSAKL |

(**): Numbering of amino acids is based on the consensus sequence generated for the studied protein. No fully conserved motifs across all RV-A (n = 75). Highlighted residues are conserved across all studied RV-A & -B serotypes (n = 100). An * (asterisk) indicates positions that have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties. A. (dot) indicates conservation between groups of weakly similar properties.

**Table 4**
Conserved motifs identified within RV-VP4 capsid protein.

| Species Group | Receptor | No. | Segment length | Region** | Consensus |
|---|---|---|---|---|---|
| **RV-A** | VP4- ICAM | 1 | 17 | 1–17 | **MGAQVSRQNVGTHSTQN** |
| | | 2 | 17 | 22–38 | **GSSLNYFNINYFKDAAS** |
| | | 3 | 16 | 46–61 | **FSQDPSKFTDPVKDVL** |
| | VP4- LDLR | 1 | 17 | 1–17 | **MGAQVSRQNVGTHSTQN** |
| | | 2 | 20 | 19–38 | **VSNGSSLNYFNINYFKDAAS** |
| | | 3 | 22 | 48–69 | **QDPSKFTDPVKDVLEKGIPTLQ** |
| **RV-B** | VP4- ICAM | 1 | 12 | 27–38 | **FTVINYYKDAAS** |
| | | 2 | 13 | 47–59 | **SMDPSKFTEPVKD** |

(**): Numbering of amino acids is based on the consensus sequence generated for the studied protein. Highlighted residues are conserved across all studied RV-A & -B serotypes (n = 100).

**Table 5**
Prediction of B-cell epitopes within conserved regions identified within VP1, VP2, VP3, and VP4 protein sequences from ABCPred Prediction Software Server.

| [22] Rank | Sequence | Start position | Score |
|---|---|---|---|
| VP4_1* | M**GAQVSRQNVGTHSTQN** | 2 | 0.64 |
| | **MGAQVSRQNV**GTHSTON | 1 | 0.61 |
| | MGAQ**VSRQNVGTHS**TQN | 5 | 0.57 |
| VP4_2* | **GSSLNYFNIN**YFKDAAS | 1 | 0.78 |
| | G**SSLNYFNINYFK**DAAS | 2 | 0.62 |
| | GSSL**NYFNINYFKD**AAS | 5 | 0.58 |
| | GSSLNYFNINYFKDAAS | – | – |
| VP4_3* | F**SQDPSKFTDP**VKDVL | 2 | 0.77 |
| | FS**QDPSKFTDPVKD**VL | 3 | 0.64 |
| VP2_1* | **GWWWKLPDAL** | 1 | 0.67 |
| VP2_2* | P**YVNAVPMDSM** | 2 | 0.55 |
| VP1_1 | ASAYYMFYDGY | – | – |

median identity of serotype pairs within group A was more than that of group B. Within the group, HRV-74 VP4 showed a median identity of 97.35% with 24 serotypes that are 100% identical. Interestingly, all the RV-A serotypes except for one (A71) show identity by 90% or more to HRV-74 VP4.

Interestingly, our current results demonstrated that HRV-74 has the highest identity and homology as compared to other HRV strains among all capsid proteins studied. This is an excellent indicator to employ such a strain in future RV vaccine development. For example, HRV-16 and -89 have been selected as the strains of choice for vaccine projects in previous studies [38]. In their studies, within-group A, the median identity of HRV-16 and HRV-89 is 70% and 67.7%, respectively. However, in our study, HRV-74 showed more than 80% identity in all capsid proteins studied. Hence, it is highly suggested that HRV-74 could be a better choice for the development of vaccines. Significant sequence variations between the four capsid proteins of the RV serotypes have always been an issue in the development of a vaccine that provides potent protection against each serotype. Therefore, a highly conserved region across a pathogen strain has always been considered to be an excellent choice to solve such an issue [39,40].

The N-terminus of VP1 and VP4 in several closely related *Picornaviruses* has been suggested to be externalized during the uncoating process. Together, they allow the viral particle to interact directly with the host cell by shaping a pore in the cell membrane, through which the viral RNA is released to the cytoplasm [41]. If this is the case, blocking such a viral site by an antibody will probably prevent the viral genome from being translocated to the host cytoplasm and, consequently, viral infectivity. Apart from that, and more interestingly, most residues of three conserved motifs

detected in RV-A VP2 are also highly conserved in RV-B. Just like VP1, two of the identified motifs are located at the VP2 N-terminus, while the third (207 PYVNAVPMDSM 217) is at the C-terminus. Unlike both proteins, the VP4 aa sequence was found to be the most conserved protein.

Interestingly, the analysis shows that 72% and 85% of the VP4 sequence is highly conserved among the HRV-A major and the minor receptor groups, respectively. HRV-B VP4, in contrast, contains less conserved regions, which exhibit only 25% of the protein's total length. Upon multiple sequence alignment of all HRV-A, three highly conserved regions were identified for VP4. The B-cell epitopes predicted from the ABCPred server could serve as potential experimental validation targets for the RV vaccine development. Therefore, our future work will be an in vivo study that involves immunogenicity and humoral responses in animal protection experiments, serologic and functional assays so as to determine the efficacy of the peptide vaccine and file a patent of the same.

## Conclusions

In conclusion, this study, therefore, identified and characterized highly conserved regions with a minimum length of 9-mers within the VP1, VP2, and VP4 protein sequences using bioinformatics. These conserved regions across the majority of RV-A, and may represent potential immune targets. Remarkably, these conserved regions have shown stability over the entire history of RV-A sequences deposited in the NCBI database, as illustrated by their low entropy values and variant frequencies. The recombinant proteins could be potential candidate vaccine against RV infection in future studies after more extensive research such as in vivo animal model testing and other procedures.

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## Competing interests

None declared.

## Ethical approval

Not required.

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