 doi:10.20944/preprints202004.0213.v1

Coronavirus ORF1ab polyprotein associated nsp16 protein is a RlmE Methyltransferase and may methylate 21S mitochondrial rRNA of host cells inhibiting protein synthesis

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SUMMARY

Covid-19 infections are rapidly spreading worldwide with more than 100000 death and thus understanding the molecular mechanism of tropism of human cells is an urgent need for drug design. We have described here a bioinformatics approach to predict the functional aspects of non-structural nsp16 protein of Corona virus. The covid-19 7098 AA large polyprotein was degraded into sixteen proteins and last nsp16 protein was found an RlmE type rRNA methyltransferase. Nsp16 has no similarity to bacterial RlmABCD but has 25 percent similarity to the bacterial RlmE protein which methylates the U2551 2-hydroxy group of Ribose. The nsp16 proteins of different corona viruses like covid-19, bat-coronavirus, SARS and MERS have strong homology. Mrm2 and Dim1 like yeast and mammalian rRNA methyltransferases have 26-33 percent homologies but not with 2-O-capping MTase as reported previously. Rrp8 MTases also has no similarity to nsp16. We postulated that mitochondrial rRNA methylation of bronchial cells were mediated by the nsp16 protein causing inhibition of protein synthesis due to poor assembly of aminoacyl-tRNA or mRNA and peptidyl transferase at the PTC. This is one of the new molecular mechanism of corona virus cellular tropism and different than ACE-2 mediated blockage of cellular signalling to inhibit aldesterone biosynthesis with abnormal Na^+ ions in cells. We also designed primers based on nsp16 cDNA sequence (nt 20659-21552, accession no MT121215) specific for Covid-19 diagnosis by RT-PCR.

KEYWORDS: covid-19, rlmE MTase, new drug target, corona diagnosis, corona pandemic

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INTRODUCTION

Coronaviruses (family Coronaviridae) are enveloped viruses with a largest positive sense, single-stranded RNA genome of 30kb (Woo et al. 2009). On genetic and antigenic criteria, CoVs have been organised into three groups: α-CoVs, β-CoVs, and γ-CoVs (Dominguez et al. 2014; Lau et al. 2015; Lu et al. 2015). Coronaviruses primarily infect birds, mammals and human, causing a variety of lethal respiratory diseases resembling the common cold, to lower respiratory tract infections such as bronchitis, pneumonia, and even severe acute respiratory syndrome (SARS). In recent years, coronaviral research must be augmented due to pandemic severe respiratory illnesses outbreaks claiming >100000 deaths (Liu et al. 2014). Covid-19 virus enter cells through ACE2 receptor-mediated endocytosis. The receptor ACE2, was abundant in lungs AT2 alveolar epithelial cell as well as cells in the kidney, heart and blood vessels (Zhao et al. 2020). One of the known regulators of endocytosis is the AP2-associated protein kinase-1 implicated novel target for therapeutic intervention. Figure-1 demonstrated the blockage of ACE-2 receptors prevents the aldosterone synthesis and thus deregulating Na+ absorbtion, blood pressure and normal renal function. We suggested a new role of cellular tropism by inhibiting cellular protein synthesis.

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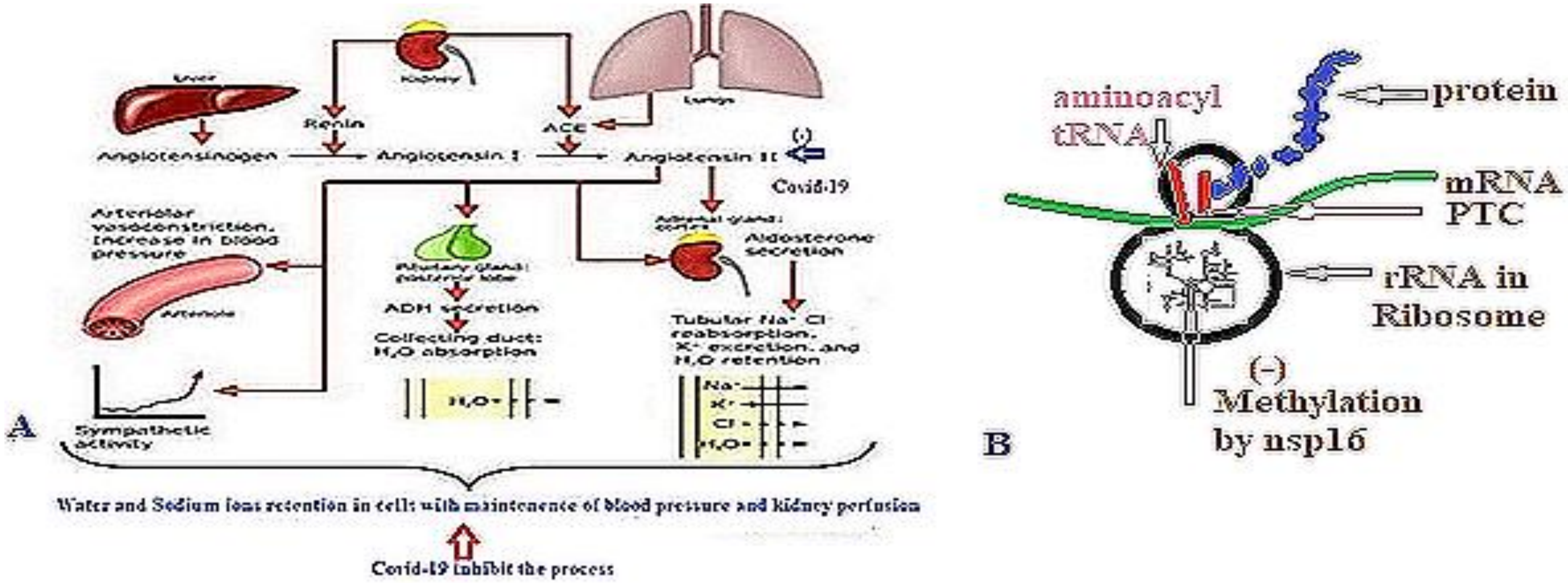

Figure 1. (A) Renin-Agiotensin System and problem associated with covid-19 binding to ACE-2 receptors preventing such signalling. (B) nsp16 methylates 21S rRNA in mitoribosome inhibiting protein synthesis. Hypothesis is due to loss of mitochondrial proteins oxidative phosphorylation may be prevented causing sudden death of Corona-infected patients.

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Discovery of new molecular target is urgent need for covid-19 and we target here rRNA methylase. There are more than 20 different classes of rRNA methyltransferases that modify 16S rRNA, 23S rRNA and tRNAs in bacteria but mammalian methyltransferases are more diverged (Bauerle et al. 2015). Cfr and Erm methyltransferases are known for erythromycin and linezolid drug resistance in bacteria and others like Rmt, ArmA and Rlm methyltransferases are involved in ribosome biogenesis as well as modulator of drug resistance (figure-2). Different isomers of Rlm (A-N) rRNA methyltransferases methylate at various positions of bacterial 23S rRNA conferring multi-resistant to macrolides and ketolides like erythromycin, telithromycin, and solithromycin. $RlmA^{II}$ MTase has preference to N_1 of G748 of 23S rRNA (Jiang et al. 2018). RlmB MTase (protein id. BAI33654) modifies G2251 of 23S rRNA (Lovgren & Wikstrom PM, 2001; Michel et al. 2002) while RlmC modifies m5U747 in 23S rRNA, and RlmD is specific for m5U1939 (Madsen et al. 2003). RlmE (protein ids. RXP80948 and TJL68081) and RlmF (prtein ids. TZE44659; BAI32636) 23S rRNA 2'-O-U2552 methyltransferase also has detected in *E. coli* chromosome and plasmids. RlmE has similarity to RlmC but diverged in other isomers. RlmG (protein id. CRY88590) methylates at N_2 of G1835, while RlmH (protein id. QBF38433) methylates pseudo-uridine N_3 at position 1915 and RlmN (protein id. QBF37927) methylates C_2 at A2503. RlmH (UbeA) catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the nucleotide at position m3ψ1915 of *E. coli* 23S rRNA (Ero et al. 2010). RlmI (protein id. QEP80348; MHW78000) also methylates *E. coli* 23S rRNA and RlmJ catalyzes the m6A2030 methylation of 23S rRNA during ribosome biogenesis in *Escherichia coli*. The active site of RlmJ with motif IV sequence 164DPPY167 is more similar to DNA m6A MTases than to RNA m6,2A MTases and the enzyme crystal structure was determined (Punekar et al. 2013). RlmM (YgdE; EC:2.1.1.186) enzyme catalyzes the SAM-dependent 2' O-ribose methylation of C2498 in 23S rRNA of *Escherichia coli* (Purta et al. 2009). RlmN installs a methyl group at the C2 position of A2503 of 23S rRNA, while it also methylates at tRNA at nucleotide A37 giving linezolid resistance in *S. aureus* (Toh et al. 2008). RlmK/L (protein ids. MHY78137 and BAI29841) and both recombined enzyme (YcbY) adds the m7G2069 and m2G2445 methylations in *Escherichia coli* 23S rRNA (Kimura et al. 2012). TlyA from *Mycobacterium tuberculosis* has proven 2′O-ribose MTase activity on C1409 of 16S rRNA and C1920 of 23S rRNA. The m^8-A2503 modification by Cfr *in vivo* leads to a decreased level of modification of C2498 by RlmM indicating domain V in presence of the Cfr-catalyzed methylation prefers a conformation where the 2′-O of C2498 is no longer accessible for RlmM. In the mature

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ribosome, A2503 stacks between A2059 and G2061 at the opposite side of the peptidyl transferase loop, and possibly the methylation, apart from sterically hindering antibiotic binding, also increases the stability of the stacked structure (Toh et al. 2008). However, ribose 2'-O-methylation similar to RlmB methylation of 2’-O of guanine, is the most abundant rRNA chemical modification to be essential for accurate and efficient protein synthesis in cells and has no role in drug resistance. Such enzymes are involved in capping of eukaryotic mRNA and modification of rRNAs and tRNAs. Human CMTr1/2 2’O-linked methyltransferase has been purified and characterized (Smietanski et al. 2014). Interestingly, Rlm MTases were rarely sequenced in plasmids of Enterobacteriaceae but in *Pseudorhodobacter sp* 1584kb unnamed plasmid indeed contained RlmF (EC:2.1.1.181) and RlmB methyltransferases and other RNA modifying enzymes (accession no. CP039965). The RlmD, RlmH and RlmN 23S rRNA Methyltransferases in large plasmid of *Acinetobacter baumannii* (accession no. CP035931, 1024kb) was reported. Surely, roles of Rlm family methyltransferases in multi-resistance remains controvertial at this point. Although it has been reported that U1939 methylation is involved in reproducible resistance to fusidic acid and capreomycin. Mitochondrial rRNA of yeast contains three modified nucleotides: a pseudouridine at 2918, two 2’-O- methylated ribose at G2270 and U2791 located near the peptidyl center. Mrm2p is the orthologue of FtsJ/RsmJ which methylates U2791 of 21S rRNA of mitochondria in yeast and mammalian cells. Di-methyl Adenine modification in yeast was mediated by Dmt1 like n6 adenine of yeast and Chlamydomonas mitochondria (protein id. EDP08643). FtsJ2 and Mrm2 methyltransferases are ivolved in 21S rRNA methylation in yeast as well as in human (Ching et al. 2002; Lee and Bogenhagen, 2014). However, some 2’-O-MTases were also implicated in capping of mRNA at the 5’-end by methyl N^7-methylguanosine linked *via* an inverted 5′–5′ triphosphate bridge to the 5′-terminal nucleoside of the transcript (Smietanski et al. 2014). Uncapped RNAs, such as nascent viral transcripts, may be detected as ‘non-self’ by the host cell, triggering an antiviral innate immune response through the production of interferons. Therefore, many viruses that replicate in the cytoplasm of eukaryotes have evolved 2′-*O*-methyltransferases (2′-*O*-MTases) to autonomously modify their mRNAs (Egloff et al. 2002; Werner et al. 2011). Virus-encoded 2’-O MTase enzymes involved in the synthesis of the RNA cap structure are different from those of host cells. As a consequence, these pathogenic cap-forming enzymes are potential targets for antimicrobial drugs (Belanger et al. 2010). Such human and rat capping MTases were distinct and no similarity to nsp16 or covid-19 RlmE MTase (Mungall et al. 2003). Thus the report on capping 2’-O methylase activity of nsp16 (nsp13 in case of SARS) is interesting

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and likely a contamination of other methylase activities (von Grotthuss et al. 2003) but nsp16 ha s no similarity to

mammalian 2'capping methyltransferase (Smietanski et al. 2014).

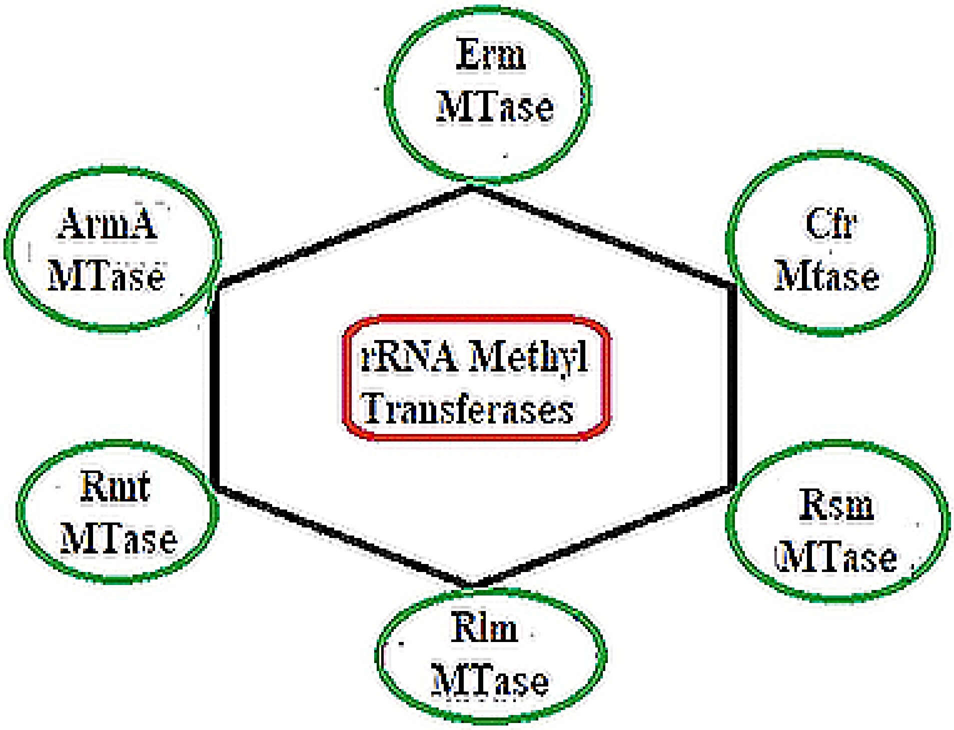

Fig.2. Different types of rRNA Methyl Transferases characterized in bacteria. But nsp16 methyltransferase is unique for Coronavirus. We propose nsp16 is rlmE type 2'0-Ribose 21S rRNA methyltransferase but not 2'-O capping mRNA methyltransferase as reported erlier.

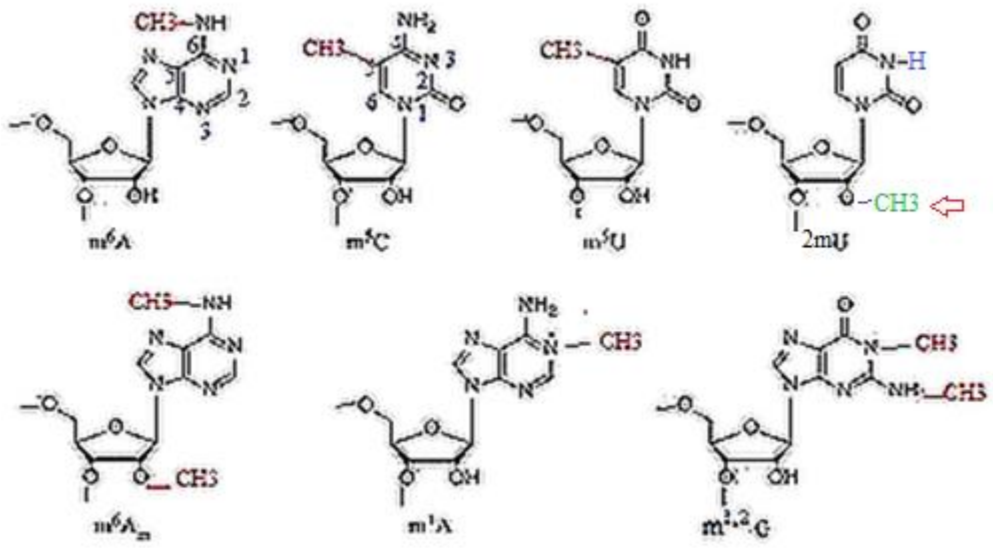

Fig.3. Methylating positions in RNA by different rRNA Methyl Transferases. Nsp16 methylates Uridine 2'-O-Ribose (green).

MATERIALS & METHODS

The BLAST search was done using web portal www.ncbi.nlm.nih.gov/blast and retrive of covid-19 and other corona viruses cDNA sequences were done using web portal www.ncbi.nlm.nih.gov/nucleotide or protein. NCBI Primer Design Software was used for primer selection and Oligoanalyzer 3.2 software was used to analyze primer dimmer and hairpin structure. Multalin Software and CLUSTAL Omega Software were used to multiple align of protein sequences and NCBI BLAST seq-2 analysis portal used to analyze homology between two sequences. NCBI pubmed portal (www.ncbi.nlm.nih.gov/pubmed) used to retrieve references and papers.

RESULT

We have analyzed the covid-19 genome and the proteins expressed by (+) sense 30kb rNA genome (Figure-4A). The polyprotein ORF1an is 7081 aa and last protein designated as nsp16 (figure4B) which has similarity to the E. coli RlmE methyltransferases which methylates Uridine^{2551} 2'-O-Ribose of 23S rRNA (figure1C). Other non-structural proteins of large covid-19 polyprotein was shown in Table-1. In Table-2, we have demonstrated the plasmid-mediated (panel-1) localization of different Rlm methyltransferases as well as other rRNA mehyl transferases (panel-3involved in drug resistance through mdr gees and drug

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efflux genes (Table-2, panel-4). The *Treponema* sp RlmE also has some homology as demonstrated in Figure-5. We searched different nsp16 proteins in variety of acute respiratory corona viruses and multialign study (figure 6A) demonstrated their homology and figure-6B demonstrated their phylogenic relation showing human covid-19 nsp16 was distinct than Avian or Feline or Canine Corona viruses. We have compared nsp16 protein with the many eukaryotic rRNA methyl transferases and found 30-33% homology at different regions showing some relations among Mrm2, Bud23 and Dim1 methyl transferases (figure-7A) with best fit region also has predicted by multi-alignment (figure-7B) but we also showed that 2'-O-Ribose Capping MTases were distinct and had no similarity to nsp16 (figure-7C). Mitochondrial Mrm2 2'-O-ribose methyltransferase required for methylation of U2791 in 21S rRNA. Such search confirmed nsp16 is a distinct rRNA methyl transferase and may have specificity to mitoribosome.

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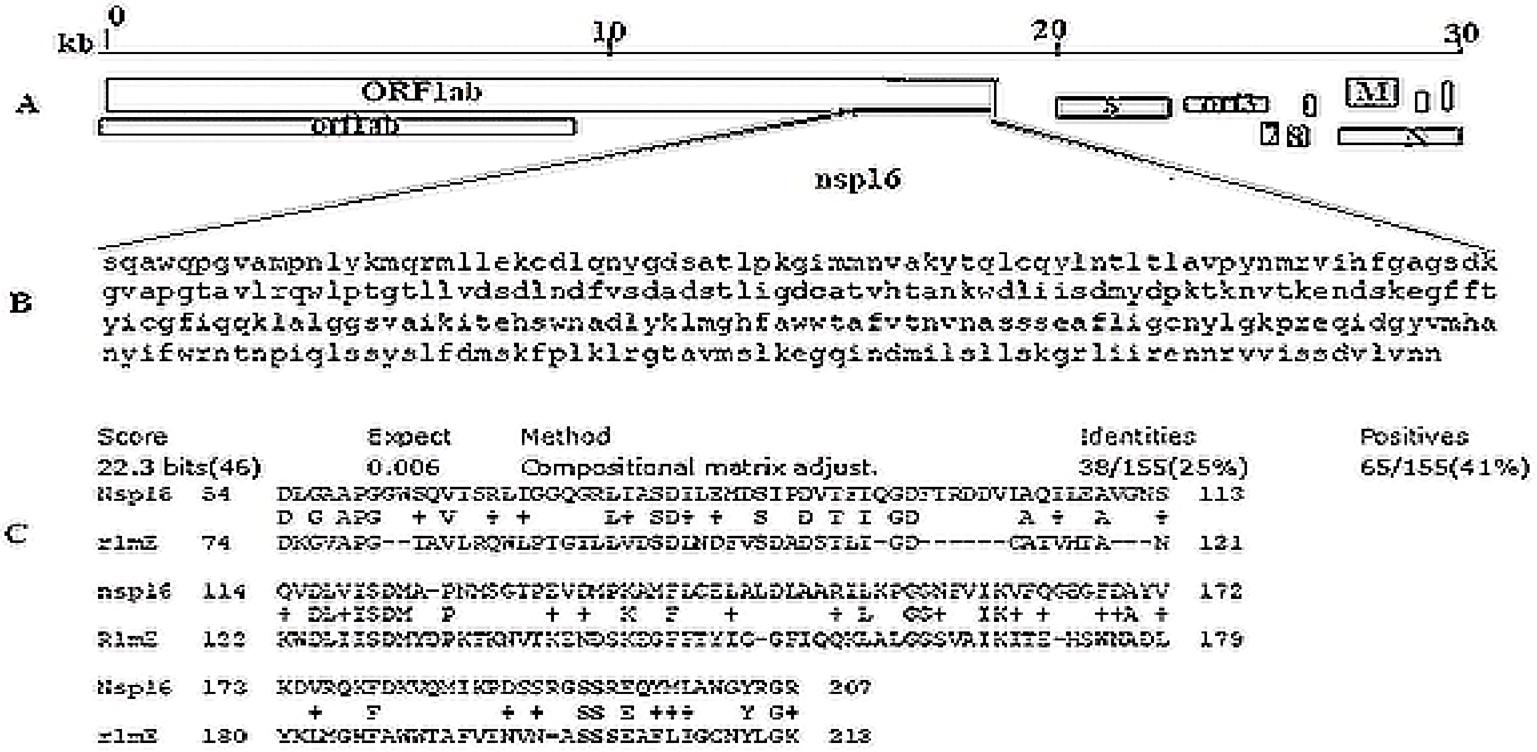

Figure 4. Localization of RlmE methyltransferases (nsp16) on the RNA geneme of Corona Virus (A), its amino acid sequence (B) and compare with bacterial RlmE protein.

Table-1: Non-Structural Proteins of Corona virus (protein id. QII57165)		
proteins	Function-1	Function -2
Nsp1	Transcription factor	Inhibits translation
Nsp2	Binds proinhibitin proteins	?
Nsp3	Transmembrane protease	ADRP activity
Nsp4, 5, 6	Transmembrane protease	protease
Nsp7	Forms hexadecameric and DNA binding	RNA polymerase open complex
Nsp8	RNA primase	?
Nsp9, 10	RNA binding	Co-factor nsp14
Nsp12	RNA dependent RNA polymerase	
Nsp13	RNA helicase	5'-phosthatase
Nsp14	Capping MTase	?
Nsp15	Endoribinuclease	
Nsp16	RNA biding	RlmE MTase

Table.2. Rmt Methyltransferases in bacterial plasmids causing alteration of ribosome structure and protein synthesis			
Bacteria types	Plasmid: size and Accession number	MTases protein id (Cfr,Erm,Arm,Dcm,Dam)	Other mdr genes, toxin genes and metal resistant genes
S. aureus	pSA-01; 63.5kb; KX274135	Cfr=ATB18028 ErmT=ATB18031 ErmB=AR044757	tetL=ARO44726 aacA-aphD=ARO44756 aadD=ARO44732
K. pneumoniae	pK-109-R; 156.5kb; KX029331	RmtG=APD70474 RsmH=APD70476; Dcm=APD70554	tetC; arr3; catB3; strB; StrA; QacE; sul1; blaCTX-M2; blaOXA1; aac6'-1b
Cronobacter sakazakii	pCsaCS931a; 4194kb; CP027108	RlmC=AXX00175 RlmK/L=AXX00103 RlmI=AXX00086	mdtA, macA/B, MFS,RND
Salmonella enterica	P2, 728kb; LN868944	RsmE/I=CRY88440/627 RlmG/M=CRY88590/328 TrmB=CRY88455	Bla_{MBL}=CRY88289; AcrE, sptP
Pseudorhodobacter sp	P1, 1584kb; CP039965	RlmF=QCO57550 RlmB=QCO57183 RsmB=QCO56847	Multidrug efflux = QCO56662, QCO57496; αβ Hydrolase = QCO58033

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(A) *Treponema* sp RlmE : Protein id. WP_010882127

```
  1 mnvykradfw akkaaaagyr arsvyklaal dkkysllsra srvldlgaap gswtqyvlgt
 61 aaactavcav dvqpiasdiq darlqrvqgd lcaadtrarv acnapfdlil sdaaprttgn
121 rtvdtsasac laagvcayvn flssdgglvf kvfqgsehla ilthlrahfg avcsfkppas
181 rprscelyvv arffrgtcgk
```

(B) Seq-2 Blast with nsp16

```
Score             Expect   Method                          Identities   Positives
26.9 bits(58)     2e-04    Compositional matrix adjust.    21/79(27%)   43%

nsp16  98   ARVACNAPFDLILSDAAPRTTGNRTVDTSASACLAAGVCAYVNF-LSSDGGLVFKVFQGS  156
            A V      +DLI+SD      T N T +  +          +C ++    L+   G +   K+ + S
rlmE   116  ATVHTANKWDLIISDMYDPKTKNVTKENDSKEGFFTYICGFIQQKLALGGSVAIKITEHS  174

nsp16  157  EHLAILTHLRAHFGAVCSF  175
             + A L   L  HF     +F
rlmE   175  WN-ADLYKLAGHFAWWTAF  192
```

Fig. 5. Seq-2 alignment between nsp16 of human Corona virus and *Treponema* sp RlmE MTase. Such little homology was not found with 20 different types of rRNA methyltransferase.

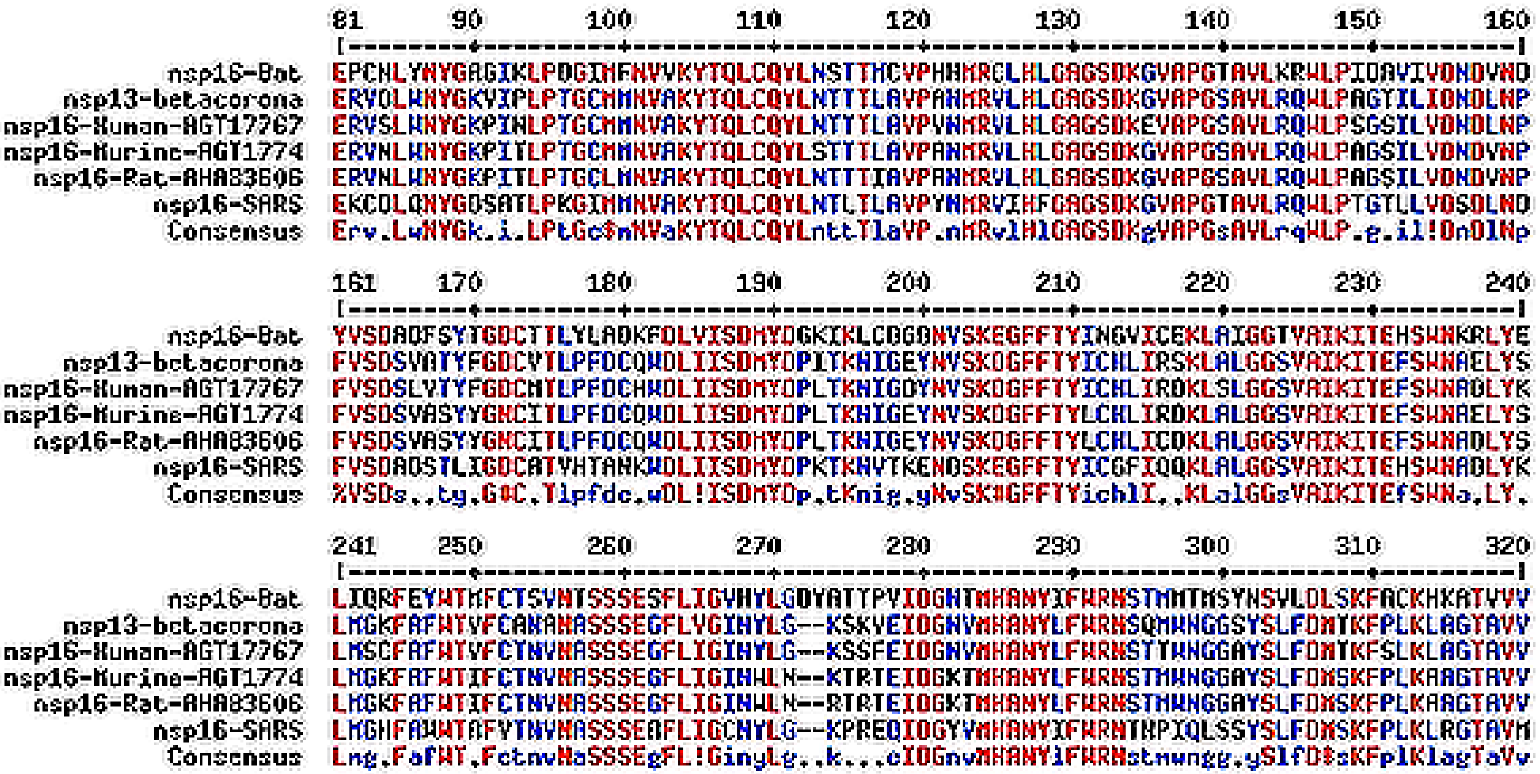

Figure 6. Multi-alignment of nsp16 proteins of different corona viruses showing strong homologies.

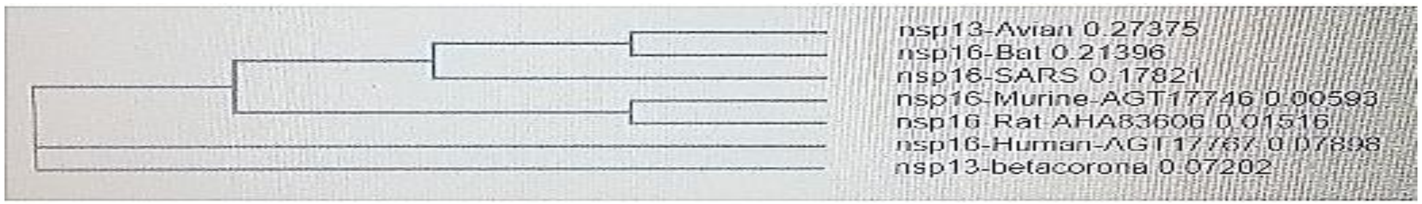

Fig.6B. Phylogenetic analysis of RlmE MTases from different species. Human coronavirus rlmE methyltransferase appeared distinct.

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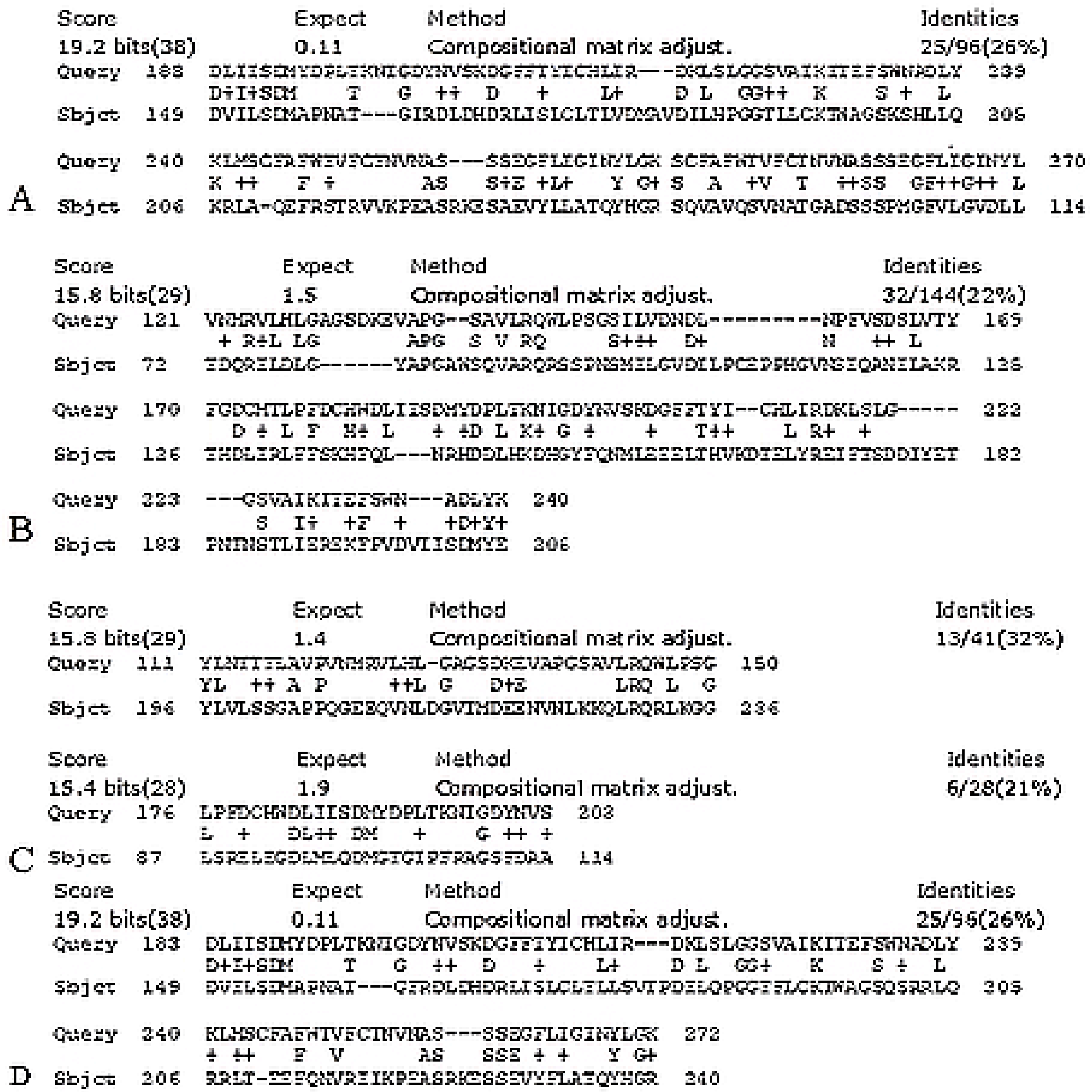

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Fig.7A. BLAST Seq2-alignment among eukaryotic rRNA Methyl Transferases and nsp16 protein of corona virus. (A) Rat RlmE-like protein (accession-NP_001100595), (B) Yeast Mrm2 MTase (accession-QHB08519) that methylates U^{2791} on 21S mitochondrial rRNA, (C) Yeast Bud23 MTase (accession-QHB07184) and (D) human Mrm2 MTase (accession- NP_037525) were compared with nsp16 of corona virus.

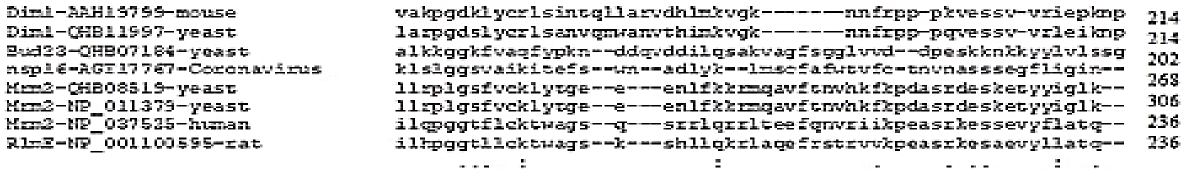

Fig.7B. Multialignment of different eukaryotic and corona virus rRNA methyl transferase showing best fit region but poor homology.

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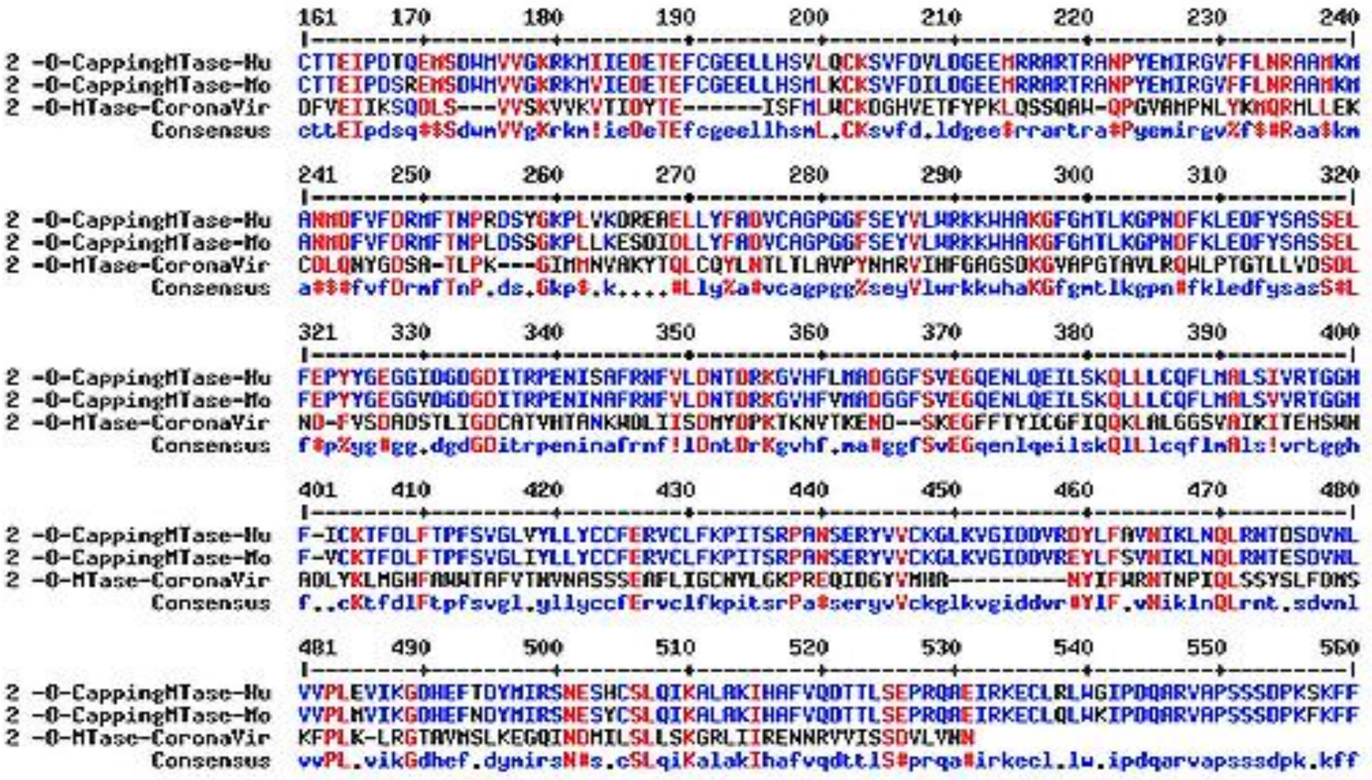

Fig.7C. No similarity of nsp16 RmtE protein with eukaryotic capping 2’-O-MTase.

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Further, we designed the covid specific primers for the RT-PCR to detect the virus load during pathogenesis. The cDNA sequence nt. 20659-21552 (accession no. MT121215) was used to design four pairs of primers using NCBI Primer Design Software (figure-8). Individual forward and reverse primer were BLAST searched and primer pair-4 appeared more specific for the diagnosis of covid-19 (figure-9). The primers were further analyzed by OligoAnalyzer 3.2 Software to check the hairpin structure (figure-10) and , Dimmer formation (figure-11). BLAST search indicated covid-19 specificity. Similarly, OligoAnalyzer analysis proved the melting point of hairpin structures varies between -12 to +23 degree centrigrade well below of Tm 60 degree centrigrade. Also dimmer formation with self or with reverse primer (pair-4) shown in figure-11 with delta-G -3 to-5 Kcal/mole reflecting a very good primer pair.GC content between 47-50% was also very good for RT-PCR as 1st strand synthesis with reverse transcriptase occurred at 48 degree centrigrade. The BLAST search of forward oligo gave 100 covid genomic sequences with 100% specificity and no human sequence was detected in such extended search with 5000 genomic sequences (figure-9). Other oligo pairs (primer pair-1) might be good to use but also hybridized to the SERS and MERS Coronaviruses (Chakraborty et al. 2020, in press). We made restriction enzyme analysis (figure-12A) showing single hexa-cutter enzymes and have chosen BglII, NheI and SspI enzymes (figure-12B) for analysis of PCR product (564bp) giving a unic pattern of agarose gel picture when restricted PCR products were compared with uncut RT-PCR product (figure-12C). This help any graduate student can see the data and can compare with own to sure that RNA is for COVID-19.

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Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm
Forward primer	TCTAGTCAAGCGTGGCAACC	Plus	20	19	38	60.32
Reverse primer	ATAGCCACGGAACCTCCAAG	Minus	20	524	505	59.46
Product length	506 bp					

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm
Forward primer	ACAATCTAGTCAAGCGTGGCA	Plus	21	15	35	60.00
Reverse primer	CGCGTGGTTTGCCAAGATAA	Minus	20	667	648	59.48
Product length	653 bp					

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm
Forward primer	AACCGGGTGTTGCTATGCCT	Plus	20	35	54	62.14
Reverse primer	TATTTGTTCGCGTGGTTTGCC	Minus	21	675	655	60.34
Product length	641 bp					

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm
Forward primer	GGGTGTTGCTATGCCTAATCT	Plus	21	39	59	57.79
Reverse primer	GTAACAAAGGCTGTCCACCA	Minus	20	602	583	58.02
Product length	564 bp					

Fig.8. Selection of nsp16 specific primer pairs for RT-PCR. Primer pair 4 was Covid-19 specific and analyzed further,

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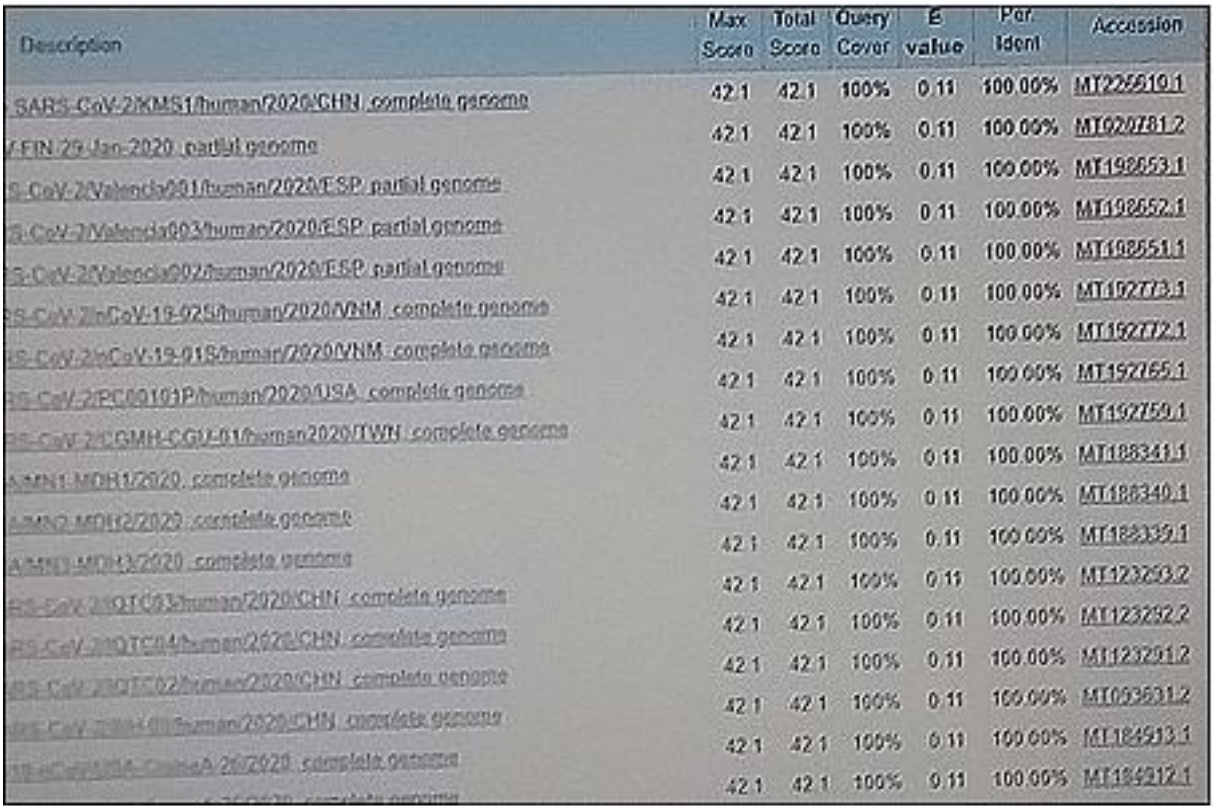

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
SARS-CoV-2/KMS1/human/2020/CHN, complete genome	42.1	42.1	100%	0.11	100.00%	MT226610.1
V-FIN-29-Jan-2020, partial genome	42.1	42.1	100%	0.11	100.00%	MT020781.2
S-CoV-2/Valencia001/human/2020/ESP, partial genome	42.1	42.1	100%	0.11	100.00%	MT198653.1
S-CoV-2/Valencia003/human/2020/ESP, partial genome	42.1	42.1	100%	0.11	100.00%	MT198652.1
S-CoV-2/Valencia002/human/2020/ESP, partial genome	42.1	42.1	100%	0.11	100.00%	MT198651.1
S-CoV-2/nCoV-19-02S/human/2020/VNM, complete genome	42.1	42.1	100%	0.11	100.00%	MT192773.1
S-CoV-2/nCoV-19-01S/human/2020/VNM, complete genome	42.1	42.1	100%	0.11	100.00%	MT192772.1
S-CoV-2/PC00101P/human/2020/USA, complete genome	42.1	42.1	100%	0.11	100.00%	MT192765.1
S-CoV-2/CGMH-CGU-01/human2020/TWN, complete genome	42.1	42.1	100%	0.11	100.00%	MT192759.1
A/MN1-MDH1/2020, complete genome	42.1	42.1	100%	0.11	100.00%	MT188341.1
A/MN2-MDH2/2020, complete genome	42.1	42.1	100%	0.11	100.00%	MT188340.1
A/MN3-MDH3/2020, complete genome	42.1	42.1	100%	0.11	100.00%	MT188339.1
S-CoV-2/IQTC03/human/2020/CHN, complete genome	42.1	42.1	100%	0.11	100.00%	MT123293.2
S-CoV-2/IQTC04/human/2020/CHN, complete genome	42.1	42.1	100%	0.11	100.00%	MT123292.2
S-CoV-2/IQTC02/human/2020/CHN, complete genome	42.1	42.1	100%	0.11	100.00%	MT123291.2
[illegible]/human/2020/CHN, complete genome	42.1	42.1	100%	0.11	100.00%	MT039631.2
[illegible]-26/2020, complete genome	42.1	42.1	100%	0.11	100.00%	MT184913.1
[illegible]	42.1	42.1	100%	0.11	100.00%	MT184912.1

Fig.9. BLAST search result of nsp16F4 oligo sequence showing covid-19 specificity.

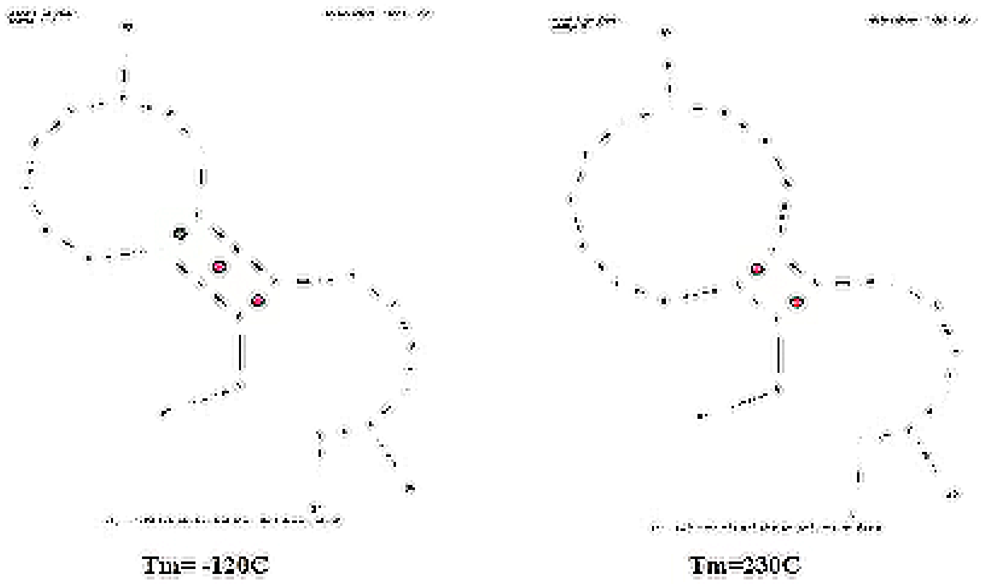

Fig.10. Hairpin formation of nsp16F4 indicating low Tm of such self complex.

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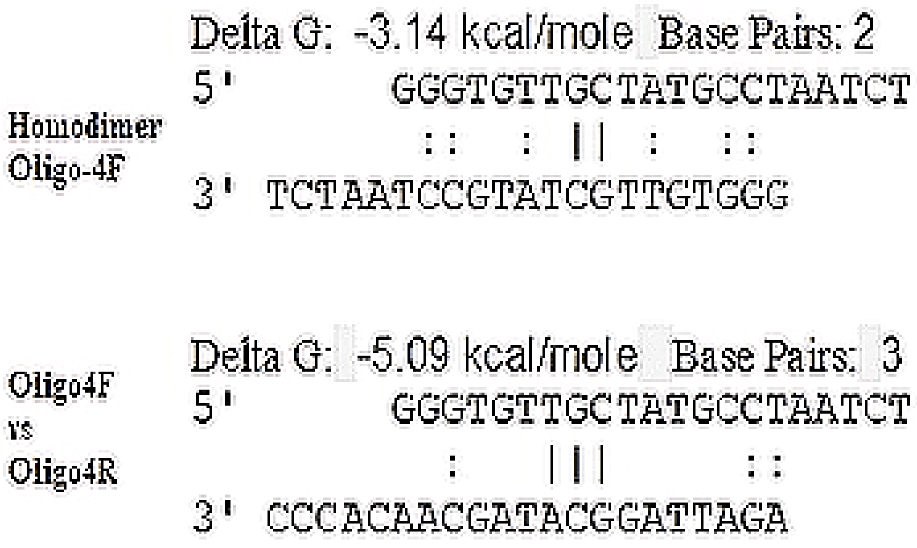
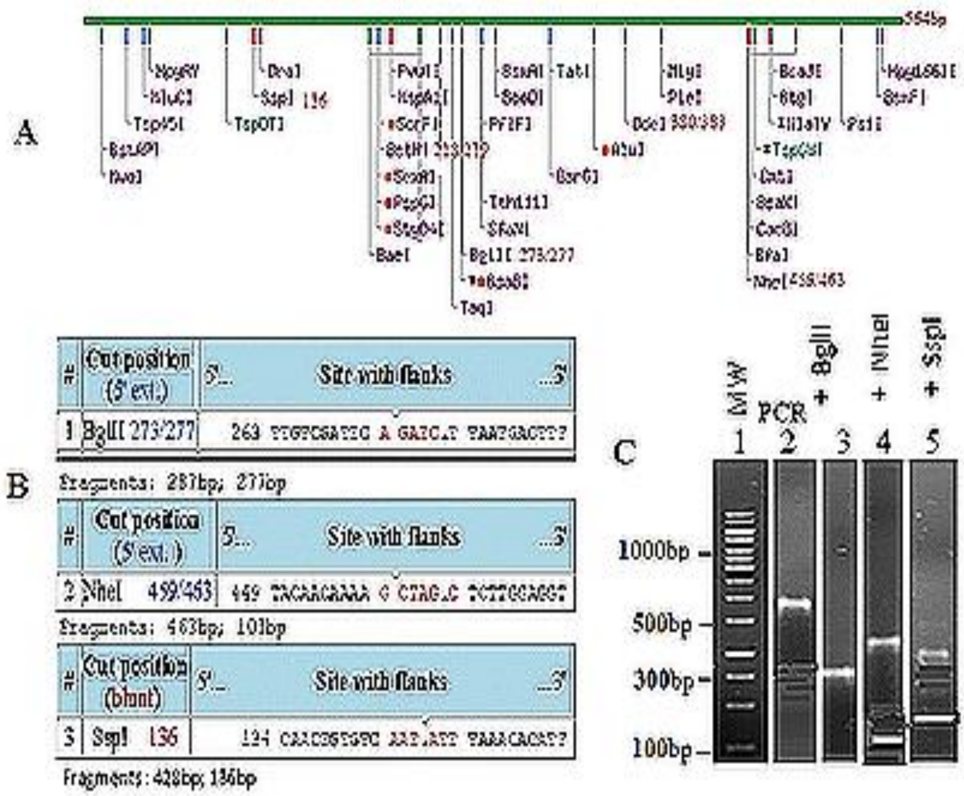


Fig.11. Dimmer formation of nsp16F4 and nsp16R4 primers showing low melting point of such dimmers. F means forward primer and R means Reverse primer. Nsp16 means coronavirus RlmE 2'-O-Ribose Uridine[2551] Methyl Transferase.



Fig,12. Analysis of the PCR product with nspF4/nspR4 primers. Restriction map of the PCR fragment (A); the 3 hexacutter single cut enzyme were chosen (B) and a 1% agarose gel in1xTAE buffer for 2hrs at 50V stained with EtBr and under UV illumination (a prototype picture was made).

DISCUSSION

We explained the possible role of RlmE methyltransferase (nsp16) in corona virus pathogenesis being a molecular target for drug design. The study is based on homology of nsp16 to bacterial RlmE methyltransferases and such enzyme was located in many coronaviruses like SARS and MERS. Viral capping MTase was shown as molecular target and thus nsp16 MTase could be an ideal target to block cellular tropism of covid-19. Three regions of ORF1ab polyprotein of covid-19 showed some homologies at the level of 20-25% (aa 1479-1569; aa3290-3360 and aa 4895-4935) with human capping MTase but not with nsp2 protein suggesting Covid-19 may code such enzyme Decroly et al. 2008; (Ferron et al. 2012). From such analysis we concluded that nsp16 RlmE was not involved in mRNA capping (Bollati et al. 2009; Zust et al. 2011). But if human mitoribosome (21S and 12S rRNAs) be methylated by nsp16 protein is an interesting question but 23S rRNA proto-type rRNA in human nucleus is 28S rRNA and some MTases (cfr and emrB) have 23S rRNA specificity rather than 16S rRNA (Figure-3).

We claim that our study is important for unknown microbe like covid-19 that is claiming >90000 lives due to lack of drug. Non-structural viral proteins are ideal for drug discovery understanding molecular mechanism of pathogenicity. It was demonstrated that P7 protein of hepatitis C virus was the target of hexamethylene amiloride (Premkumar et al. 2014) where as the rotavirus NSP4 viroporin domain was shown as calcium-conducting ion channel (Pham et al. 2017). Coronavirus envelope protein or nucleocapsid protein have shown as molecular targets (Schoeman & Fielding , 2019). *Nigella sativa*, *Anthemis hyalina* and *Citrus sinensis* phytochemicals have some antiviral effect on Corona viruses replication (Ulasli et al. 2014). Such anti-viral drug screening against covid-19 is an urgent need to control deadly pandemic nature of pathogenesis (Kilianski &, Baker, 2014). Various host factors have been postulated in the literature and need more elaborate study for drug design (Just et al. 2011; de Wilde et al. 2018). Targeting cellular autophagy may be one such application to control covid-19 mRNA genome release into cell cytoplasm (Prentice et al. 2004). Natural products have shown to regulate microbes and became good drugs controlling superbug bacteria, malaria and cancer (Chakraborty, 2019; Newman & Cragg , 2012; Chakraborty, et al. 2018). The 7a protein of Coronavirus has shown to induce apoptosis by caspase-dependent pathways and thus antibody against 7a protein may be a drug against viral pathogenesis (Tan et al. 2004). Interferon-alpha2b and ribavirin improve outcome in MERS-CoV-infected rhesus macaques giving a hope for the treatment of Covid-19 (Falzarano et al.2013).

We have clearly demonstrated that nsp16 is a RlmE type methyl transferase that may methylates 21S rRNA ofmitochondria but may be 28S rRNA of human bronchial cells also be methylated by nsp16 protein. Bronchial cells with ACE-2 receptors are target for Covid-19 and methylation of rRNAs in such target cells as well as kidney and heart cells may be possible causing abnormal protein synthesis. Such methylation may favours viral protein synthesis inhibiting cellular protein synthesis that have shown in many viral infections (figure-4). In truth stopping Rennin-Angiotensin pathway (figure-1) for aldosterone synthesis causes abnormal Na^+ ions in cells lowering blood pressure and kidney function. Covid-19 RlmE type MTase has never be implicating in pathogenicity and thus we have discussed a new direction of cellular tropism of Corona viruses. Nsp16 MTase has some similarity to the mmr2 and Dim1 rRNA methyl transferase of yeast and mammals (figure-7). Yeast Rrp8 23S rRNA MTase has poor similarity to nsp16 but no similarity with human Rrp8 protein (data not shown). The primers designed (figure-8) using Coronavirus *rlm*E gene sequence may be used for diagnosis and therapeutic discovery in the future. Heterogeneous phyto-antibiotics, gene therapy, anti-sense and ribozyme technology and nano-drug carriers may be future medicine against Covid-19 (Cao et al. 2015; Chakraborty, 2019).

ACKNOWLEDGEMENT

I thank Dr. Parthapratin Chakravorty of Raja N L Khan Women's College for encouraging covid-19 research.

CONFLICT OF INTEREST

No conflict of interest. This is lockdown research from home.

ETHICAL ISSUES

No patient was selected in this bioinformatics research

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