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COVID-19 Pandemic and the Global Vaccine Strategy

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ABSTRACT

The vulnerability of an innumerable number of people worldover to COVID-19 is still obvious though it has been well over an year since the virus first emerged. The humanity has faced second wave of infection by SARS-CoV-2, and it has been a way of life by now to change our life styles, particularly adopting work from home, or simply staying back at home for the students pursuing studies at Colleges and Universities or the minors at primary institutions. These methods have been adopted to break the chain of virus that could reduce the opportunities for the virus to spread. Vaccines leave an imprint on our bodies to enable them to fight the infection and are "the" exit strategy from the pandemic. The current paper reviews various types of vaccines and the recent advancements made to fasttrack preparations of a wide variety of newer vaccines that could be economical or may take the form of a capsule or similar other variant. The efficacy of the two Indian vaccines, Covaxin and Covishield are compared.

Keywords: SARS-CoV-2, Covaxin, Covishield, RNA vaccine, inactivated virus.

INTRODUCTION

COVID-19 is a recent disease, that has occurred due to the SARS-CoV-2 virus, though its origin has been uncertain. A fever, dry cough, tiredness and loss of taste or smell are the key symptoms of COVID-19. This virus bears the potential to infect human beings through a variety of channels. Some people do not have any symptoms and may not even know they have the virus, while others get seriously ill and need hospital care. The infection by this virus can be prevented by applying Covid appropriate behavior incorporating wearing a mask, maintaining social distance of 6ft as well as frequent handwashing.

The spread of Covid-19 is usually transmitted by a close contact with the person afflicted by the disease. The non-infected person is likely to breathe in the respiratory droplets falling out of the mouth and nose of the person that harboured virus. The groups of people staying together for longer period of time indoor *viz.,* crowded and poorly ventilated spaces, such as restaurants, gyms, nightclubs, offices and places of worship. The non-infected person could also likely come into the contact of respiratory droplets of the infected person when he speaks or sings, in addition with the coughs and sneezes that could land on surfaces, like handles of doors, table surface, backrest of chairs, metal railings of staircase etc. The virus is then spread when another person comes into contact with these droplets and touches their own eyes, nose or mouth, without washing his/her hands first. It is not a sexually transmitted infection, however, it can be passed on through kissing and close contact, including having sex. The kissing or practicing sex should be avoided with a partner that manifested COVID-19 symptoms. Sexual health services, including for family planning, contraception and pre-exposure prophylaxis (PrEP), may be disrupted by the impact of COVID-19.

Though any person could be susceptible to COVID-19 infections and could fall sick seriously, the aged persons beyond 60yrs of age, including those with co-morbid situation like high blood pressure, heart or lung problems, diabetes or cancer are at a higher risk of developing serious

illness. People living with HIV who have a compromised immune system – those with a low CD4 count, a high viral load or recent opportunistic infection – are also more at-risk. However, evidence is lacking on the increased risk to contract COVID-19 besides developing serious symptoms, to the people living with HIV as well as on effective antiretroviral treatment.

A vaccine works by assisting a human body to develop immunity against SARS-CoV-2 or, for that matter, any other microorganism that is pathogenic. This would mean that the human body would be well prepared, on exposure to the pathogen, to respond to it and no serious illness is likely to affect the body. The efficacy and safety perception of the vaccine can only be acknowledged when it has undergone strict clinical safety trials before these are approved by National Regulators, the Drugs Controller General of India (DCGI), in case of India. The subjects even after having received vaccine would have to follow prevention measures such as social distancing and wearing a mask until the number of people with COVID-19 falls to a safe level in a particular area.

A vaccine is a substance that is introduced into the body to prevent infection or to control disease due to a certain pathogen (a disease-causing organism, such as a virus, bacteria or parasite). The vaccine "teaches" the body how to defend itself against the pathogen by creating an immune response. The immune response by a biological agent defends human body against an invading pathogen (virus, parasite or bacteria). Such biological agent is termed as Vaccine. Usually, its liquid form is effective in our body, either by injection, by oral doses, or by intranasal routes. India has now introduced a newer capsule form of its indigenous Covaxin, which is under process of development in collaboration with Israel.

The variety of vaccines, their types and sources are summarized in Table 1. The sites of mutation of each variant being encountered in Indian patients as well as the Indian Double Mutant with their respective locations are listed in Table 2.

Specifically UK Variant B.1.617 has been detected in Punjab, while this variant has also been detected in Delhi along with certain other variants that are being still searched. On the other hand, this UK variant has been occurring in some cities of Maharashtra. Genetic maps of novel lineages incorporating Double mutant, Triple mutant as well as Bengal variant has been depicted in Fig. 1.

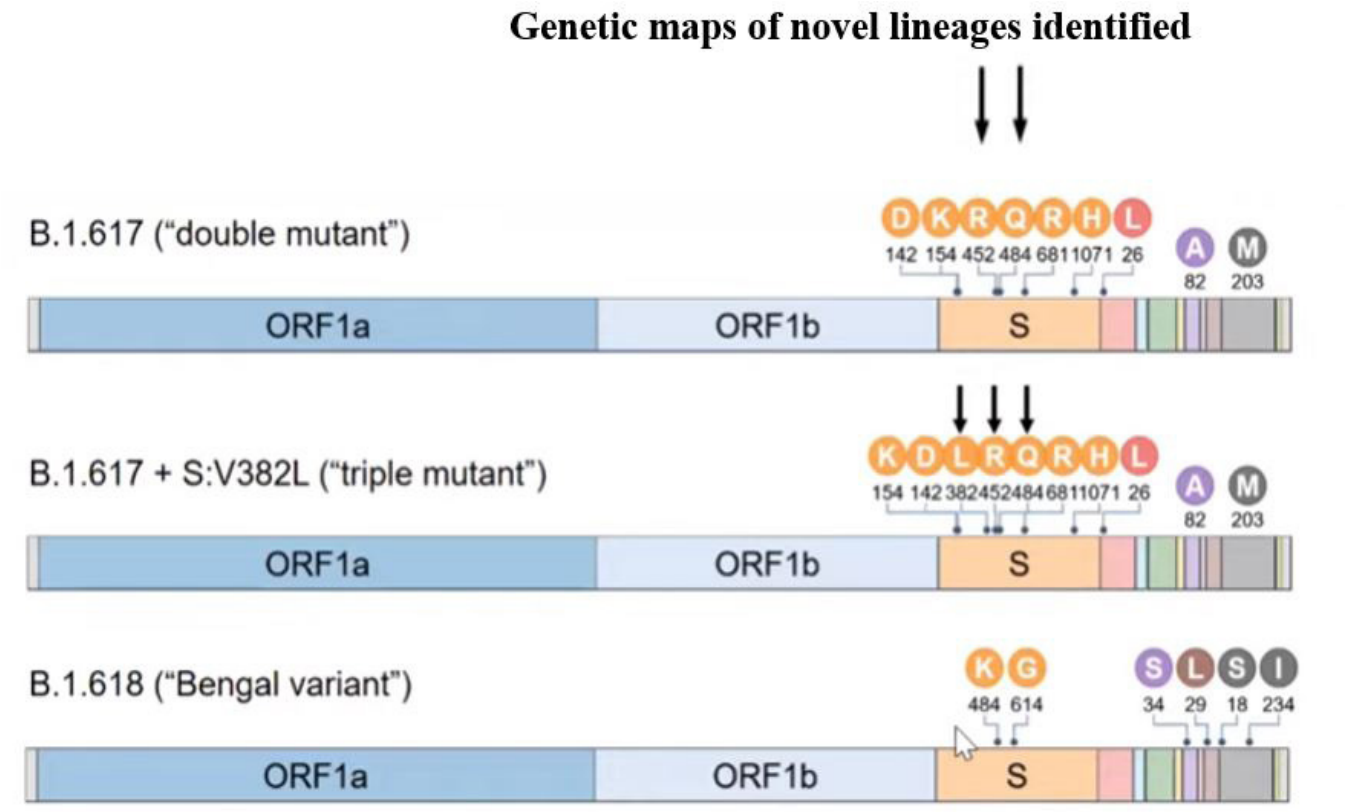

Fig. 1. Genetic mapping of different variants spread over different states in India.

Mutations

- A mutation is a change in the genetic material of the virus when it replicates.
- Mutations happen by chance, but selected based on fitness
  - Advantageous for the virus – represented more in the samples
  - Disadvantageous – selected against and wiped out from the population.

More mutations could often be more dangerous

SARS-CoV-2 mutates approximately once in two weeks.

Key Mutations

The following are the key mutations that corona virus has undergone since the time it arrived in India:-

- **L452R increases receptor binding and decreased sensitivity to some neutralizing antibodies**
- **E484K/Q increases receptor binding and reduces efficacy of vaccinated sera and antibodies**
- **L452R+E484Q unique combination in India**
- **N501Y increases receptor binding and alters antibody recognition**
- **N440K immune evasion: loss of binding to one neutralizing antibody.**

Mutant and Variant Strain

Any changes in the viral genetic sequence during replication is known as a mutation and viruses with new mutations are sometimes called variants. Variants can differ by one or multiple mutations.

When a new variant with one or multiple mutations shows different functional properties to the original virus and becomes established in a population, it is sometimes referred to as a new strain of the virus.

All strains are variants, but not all variants are strains.

What are variants and lineages

- Variants are viruses with one or more mutations compared to the reference
- A lineage is a set of viruses sharing a common ancestor
- **UK Variant (B.1.617)**
  50% increased transmission
  Increased severity
- **South African Variant (B.1.351)**
  50% increased transmission
  Reduced antibody neutralization
- **Brazil Variant (P.1)**
  Reduced neutralization by antibodies
  Reduced neutralization after vaccination

Variant of Interest: changes in receptor binding, reduced neutralization by antibodies generated against previous infection or vaccination, reduced efficacy of treatments, potential diagnostic impact, or predicted increase in transmissibility or disease severity.

Variant of Concern: There is evidence of an increase in transmissibility, severe disease severity, reduced effectiveness of treatments or vaccines, or diagnostic detection failures.

Variant of High Consequence: There is clear evidence that prevention measures or medical counter measures (MCMs) have significantly reduced effectiveness relative to previously circulating variants.

What might be a variant of interest (VOI**)?**

- Mutation likely to affect receptor binding, reduced neutralization.
- Mutation predicted to affect transmission, diagnostics, therapeutics, or immune escape.
- Evidence that it is the cause of an increased proportion of cases or unique outbreak clusters.
- Limited prevalence in population.

Name of Variant	Country of Origin
B.1.617 or "Double Mutant"	- Indian variant carries two mutations: L452R and E484Q
B.1.526	- NY with E484K

What might be a variant of concern (VOC)?

- Mutation that increases virus transmissibility,
- Virus causing more severe disease reflected in increased hospitalizations or deaths,
- Virus with significant reduction in neutralization by antibodies generated during previous infection or vaccination,
- Virus causing reduced effectiveness of treatments or vaccines,
- Virus escaping diagnostic detection

Name of Variant	Country of Origin
B.1.617	- UK
P.1	- Japan/Brazil
B.1.361	- SA
B.1.427	- California

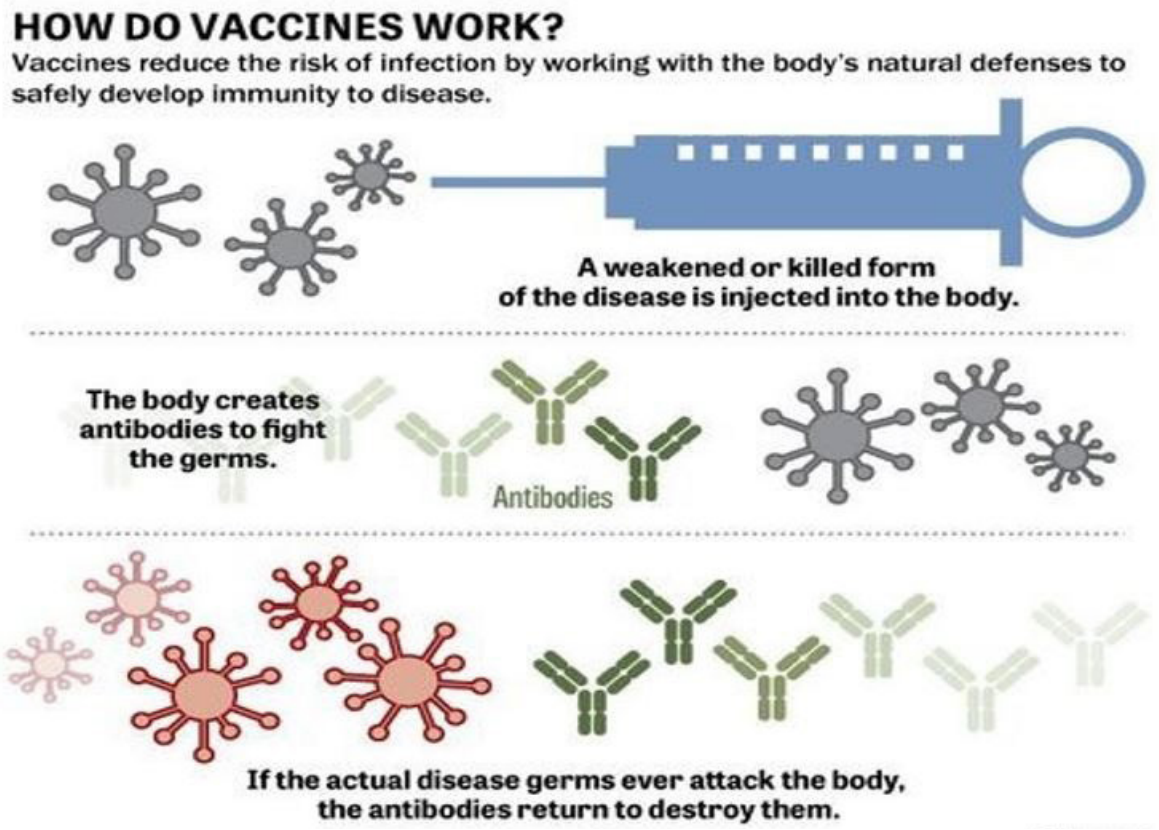

Fig. 2. The mechanism of functioning of vaccines.(after Klingensmith, M. 2021)

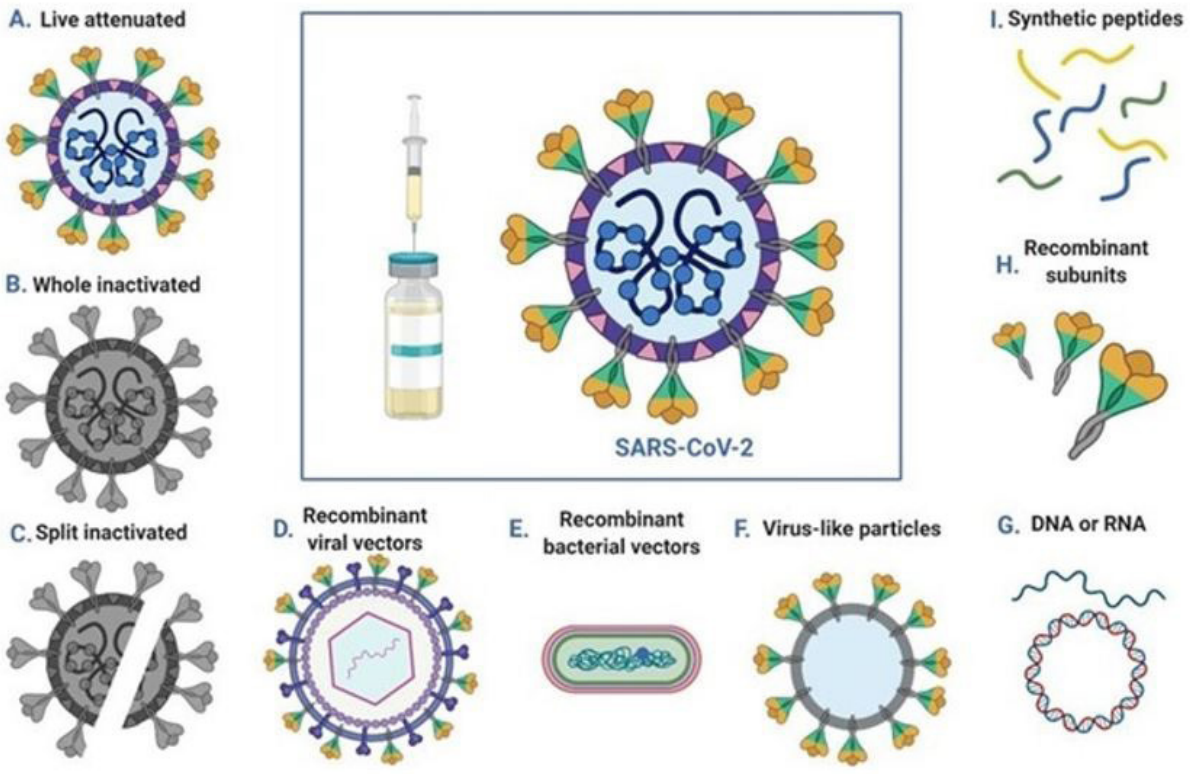

Fig. 3. The constituents of COVID-19 vaccine in India.

How do vaccines work

A preventive vaccine protected an individual from infection or disease in the following manner (Fig. 2):-

1. The vaccine introduced a small component or a non-harmful form of the pathogen into the body. This is called the foreign antigen or immunogen.
2. The body's immune system, in turn, produced an immune response to the pathogen by generating antibodies, killer cells, or both.
3. A small group of "memory" B-cells and T-cells remain in the body thus could quickly initiate a strong immune response, *i.e.,* by producing antibodies, and helping the production of killer T-cells or antibodies, respectively. The next time the real pathogen entered the body, the immune system remembered it and mounted a much larger, quicker response than it would have, if the individual had never received the vaccine.

In order to respond quickly and effectively to the COVID- 19 Pandemic, a broad range of candidate COVID-19 vaccines are being investigated globally using various technologies and platforms. These include Viral – Vectored, Protein subunit, Nucleic acid (DNA, RNA), Live attenuated and Inactivated vaccines.

Types of vaccine:

The classification of vaccines depended on the way these were synthesized (Fig. 3).

1. Live attenuated vaccines

Pathogens, like virus or bacteria are weakened by genetic manipulations to limit their growth and thus failed to cause disease to the host. In some modified versions of live vaccine, an organism that is related to the pathogen, is used that naturally grows poorly in humans. The weakened pathogen generated a broad immune response in the host similar to that shown by an infected individual with a natural pathogen.

Examples:

i) Oral Sabin polio vaccine
ii) MRV Vaccine (Measles, Mumps, Rubella, and Varicella)
iii) Nasal influenza vaccine
iv) Bacille Calmette-Guerin (BCG) vaccine
v) Varicella vaccine
vi) Rotavirus vaccine

2. Inactivated or Dead vaccines

The disease-causing pathogen is killed or inactivated, usually through a thermal (application of high temperature) or chemical (formalin etc.) process. Such vaccines, when administered, elicit a robust immune response that mimicked most of the responses seen during an infection.

Examples:

i) Typhoid vaccine
ii) Influenza vaccine
iii) Salk polio vaccine
iv) Hepatitis A vaccine

3. Acellular or Subunit vaccines

These are devoid of whole cells, and do not contain the whole bacteria or viruses. Instead, they contain polysaccharides or proteins from the surface of the bacteria or virus. These polysaccharides or proteins are the parts that our immune system recognizes as 'foreign' and evoke immune response against them. There are many types of acellular vaccines:

A. Toxoid Vaccine The toxins or poisonous proteins are released by several pathogenic bacteria while they attack the body. Toxoids are thus the vaccines prepared chemically by chemical inactivation of these toxins. Although they appeared like toxins yet were themselves non-poisonous. However, a stronger immune response emanated from them.

Examples:

i) Diphtheria vaccine
ii) Tetanus vaccine
iii) Pertussis vaccine

B. Conjugate Vaccine In earlier decades, the sugar molecules that occurred on the surface of bacteria were utilized to prepare Polysaccharide Vaccines. Their efficacy on infants and younger children was poorer. However, the conjugation of bacterial polysaccharide molecules or their effective chemical linkage with a carrier protein could trigger working of such vaccines in a better fashion. Further, if additional proteins were added, the immunological attributes of the carrier were transferred to the antigen, and thus a stronger immune response was induced that improved efficacy of this vaccine in younger children too.

Examples:

i) Haemophilus influenza type b (Hib) conjugate vaccine
ii) Pneumococcal conjugate vaccine
iii) Meningococcal C conjugate vaccine

C. Recombinant Vaccine A small piece of the DNA is taken from the disease-causing bacterium or virus. The particular gene is incorporated into plasmid or a carrier

vehicle which enabled production of large quantities of well-defined proteins, which were then used as vaccines.

Examples:

i. Hepatitis B vaccine

ii. Human papilloma virus (HPV) vaccine

D. DNA/RNA Vaccine Genetic material, either DNA or RNA, from the pathogenic bacteria or virus is introduced into the human cells and then the cell machinery is employed to produce the protein encoded by the inserted gene(s) of the pathogen. Our body's immune system detects such protein as a foreign agent and produces an immune response against the whole pathogen. At present, different types of nucleic-acid vaccines are in developmental, pre-clinical and clinical evaluation phases e.g. HIV vaccine

Status of the COVID-19 vaccine in India

Globally there have been more than 100 COVID-19 vaccine development projects underway, with several candidates already being tested on humans. The vaccines of Indian origin being tested on human beings are listed as below:

COVAXIN:

Developed by Bharat Biotech in collaboration with the Indian Council of Medical Research (ICMR) and National Institute of Virology (NIV) this indigenous, inactivated vaccine is manufactured in Bharat Biotech's BSL-3 (Bio-Safety Level 3) high containment facility. After approval from Drug Controller General of India (DCGI) for Phase I, II, III Human Clinical Trials, 91% success was registered in Phase III trials. A quality comparison of the two indigenous Indian vaccines, Covaxin and Covishield is presented in Table 3. Recently, in appreciation of the efficacy of the Indian indigenous vaccine, Covaxin, the characteristic to neutralise the 617 variant of the deadly virus, SARS-CoV-2 was highlighted by the White House chief medical adviser and America's top pandemic expert Dr Anthony Fauci. His observations were based on convalescent Sera of COVID-19 cases and people who received the vaccine, Covaxin used in India. Thus, this vaccine was termed as the very important antidote against the deadly Coronavirus. It was emphasized that Covaxin worked by teaching the immune system to make antibodies against the SARS-CoV-2 coronavirus. The antibodies attach to viral proteins, such as the so-called spike proteins that stud its surface. Covaxin was approved for emergency use on January 3. The proven efficacy of Covaxin, 78% has been proven.

Covishield

The Serum Institute of India (SII) and ICMR jointly conducted Phase II/III, Observer-Blind, Randomized, Controlled Study to determine the safety and immunogenicity of Covishield (COVID-19 Vaccine). Approval for Phase I, II, III Human Clinical Trials was received from DCGI and finally all trials have been completed.

AstraZeneca's ChAdOxnCoV-19

The University of Oxford took advantage of its immense experience with the nonreplicating ChAdOx1 vector, and joined AstraZeneca and Serum Institute of India, to develop ChAdOxnCoV-19 which expressed a full length wild type version of the spike protein. Initially their preliminary results from a Phase I/II single-blind randomized control trial in 1077 participants aged 18-55 were reported . In the vaccine group, a larger chunk of participants were given a single dose of 5x105 VPs, and a smaller cohort of 10 individuals were also provided a booster dose 28 days post-prime.

A meningitis vaccine was used in the placebo control group which allows for comparisons of the safety profile with a licensed vaccine. Antibody responses were tracked using several binding assays as well as three different neutralization assays, all performed with authentic SARS-CoV-2. Cellular immune responses were measured using an IFN-γ ELISpot with PBMCs stimulated using a peptide pool spanning the S. To determine neutralizing antibody responses, a subgroup of 35 individuals was analyzed. Using a 50% plaque reduction neutralization titer (PRNT50) assay, a microneutralization (MN) assay with IC80 as readout and a virus neutralization assay based on CPE 28-day post vaccination titers were 1:218 (median titers, 100% seropositivity), 1:51 (median titer, 91% seropositivity) and in the 1:4-1:16 range (62%, this assay measures potentially an equivalent to IC100), respectively. A booster dose increased the titers in the latter two assays to 1:136 (100%) and 1:29 (100%). Of note, pre-existing immunity to SARS-CoV-2 was found in a small number of participants (4%). Cellular immunity peaked at day 14 with 856 SFU per 106 cells and waned to 424 SFU by day 56. Background cellular immunity was found mostly in the 50-100 SFU per 106 PBMCs range. Fatigue (>70%) and headache (>60%) were the side effects frequently noticed. The common symptoms were a high body temperature or feverish body. The booster dose seemed to be better tolerated but the sample measured was too low (WHO, 2020). Overall, ChAdOxnCoV-19 had a worse safety profile than the licensed meningitis vaccine used in the placebo arm, independently if paracetamol was given to alleviate side effects or not. Phase III clinical trials of this Vaccine have been completed in South Africa, Brazil and UK as one-dose or two-dose regimen (ISRCTN89951424, NCT04516746).

Coronavirus vaccination-Dose Interval

Many studies have shown that when the vaccine is administered with a gap of 6-8 weeks between the two doses, the protection is enhanced. In a meeting, the National Technical Advisory Group on Immunization (NTAGI) and National Expert Group on Vaccine Administration for COVID-19 (NEGVAC) have agreed to increase the gap between the two doses on the backing of scientific evidence. After recommendations were made, the Union Health Ministry has also agreed to it. However, this is only applicable for Oxford-AstraZeneca's Covishield and not Bharat Biotech-ICMR's Covaxin.

Now, as per the recommendations, the second dose which earlier used to be administered at 4-6 weeks can be stretched till 6-8 weeks. Union Health Secretary Rajesh Bhushan in a written letter informed the Chief Secretaries of States/UTs regarding centre's go-ahead for increasing the interval between two doses of the COVID-19 vaccine. The ministry said "it has accepted the recommendations made by NEGVAC and NTAGI." It added that as per the new guidelines, states and UTs can administer the second dose of Covishield to beneficiaries within the 4-8 weeks after the first dose is given.

Pfizer's BNT162b1 and BNT162b2

Pfizer, in collaboration with the German company BioNTech, published data from an the Phase I/II randomized, placebo-controlled, observer-blind dose escalation study with BNT162b1 in 45 healthy adults, 18-45 years of age (NCT04368728)60. BNT162b1 is an mRNA-based, LNP delivered vaccine, that expressed a trimeric version of the RBD that is held together by a T4 foldon. Three doses, 10, 30 and 100ug of RNA were tested in a prime-boost vaccination regiment with a 3 week interval. ELISA binding to RBD and neutralization of a SARS-CoV-2 reporter virus (IC80) was tested. Three weeks post dose 1, neutralization titers were in general low (similar to the mRNA-1273). Seven days post dose 2, GMTs of 1:168 and 1:267 were detected (the 300ug group was not boosted due to an unfavorable safety profile). At 14 days post boost titers reached 1:180 and 1:437, respectively. Convalescent serum was tested side by side and reached 1:94.

The trial also included a group of older individuals (65-85 years). Reactogenicity for both vaccines was lower in this group compared to younger individuals but antibody titers were also lower (GMTs at approximately 40% of the younger individuals) . BNT126b2 was selected to move forward and is now in a Phase III study in healthy adults and the elderly (NCT04368728).

ZyCoV-D

Novel Biologicals, Biosimilars and Vaccines, announced its plasmid DNA vaccine, ZyCoV-D, to prevent COVID-19. Zydus Cadila, focused on discovering and developing New Chemical Entities (NCEs). Safety in Phase I clinical trial of ZyCoV-D in healthy subjects established as endorsed by the independent Data Safety Monitoring Board (DSMB). Zydus Cadila received approvals from the DCGI to start Phase III. The company will now be initiating Phase III clinical trial in around 30,000 volunteers. ZyCoV-D was found to be safe, well tolerated and immunogenic in the Phase I/II clinical trials.

Sputnik V

Dr. Reddy's Laboratories Limited, Hyderabad and Sputnik LLC, Russia are jointly conducting Multi-centre, phase II/III adaptive clinical trial to assess safety and immunogenicity of Gam-COVID-Vac combined vector vaccine. The vaccine received approval from DCGI and they are ready with the vaccine after successful Phase III clinical trial.

Biological E's novel COVID-19 vaccine

Biological E. Limited is conducting a prospective open-label randomised Phase-I, seamlessly followed by Phase-II study to assess the safety, reactogenicity and immunogenicity of Biological E's novel COVID-19 vaccine containing Receptor Binding Domain of SARS-CoV-2 for protection against COVID-19, when administered intramuscularly in a two-dose schedule to healthy volunteers.

HGCO19 mRNA vaccine

Gennova, Pune supported with seed grant under the Ind-CEPI mission of Department of Biotechnology of M/o Science & Technology has received approval from Indian drug regulators to initiate Phase I/II human clinical trial.

The Forthcoming Initiatives:

a. VINCOV-19

The initiative by C.S.I.R. and the University of Hyderabad to utilize the conventional technique by which the antivenom is being prepared since ages has been appreciated worldover. The invasive corona virus is introduced in inactivated form, like that utilized to prepare COVAXIN, along with an adjuvant, intravenously into the horse's body, and this triggered production of antibodies against the virus. These antibodies are proposed to be collected to produce vaccine named, VINCOV-19 by the conventional method. The Drugs Controller General of India (DCGI) has also acknowledged approval to conduct clinical trials using this vaccine.

b. Nasal Vaccine by Bharat Biotech:

The most dangerous situation of serious concern is the

changing scenario of corona virus that has rapidly mutated since the time second wave started. Therefore, a variety of variants being regularly generated have created a dreadful situation for human beings. It has been reported to have the potential to neutralize the deadliest variant B.1.617. In comparison to other vaccines from developed and other countries abroad, only a single dose of this vaccine would be required instead of the double dose, that is the requirement of other vaccines. The vaccine has reached at the 3rd stage of development by Bharat Biotech, Hyderabad, and hopefully very soon it will be rolled out for public use.

c. India registers progress to develop a Capsule to replace injectible Vaccine

An Oral Vaccine is nearing completion to be developed by the Indian Company Premas Biotech in collaboration with the Israeli Company Ormed (Koshy, 2021). It has reached the stage of IInd trial. This is being considered a landmark development (Fig. 4) after India has attained success to develop 2 vaccines, Covaxin and Covishield.

In order to respond quickly and effectively to the COVID- 19 Pandemic, a broad range of candidate COVID-19 vaccines are being investigated globally using various technologies and platforms. These include Viral – Vectored, Protein subunit, Nucleic acid (DNA, RNA), Live attenuated and Inactivated vaccines.

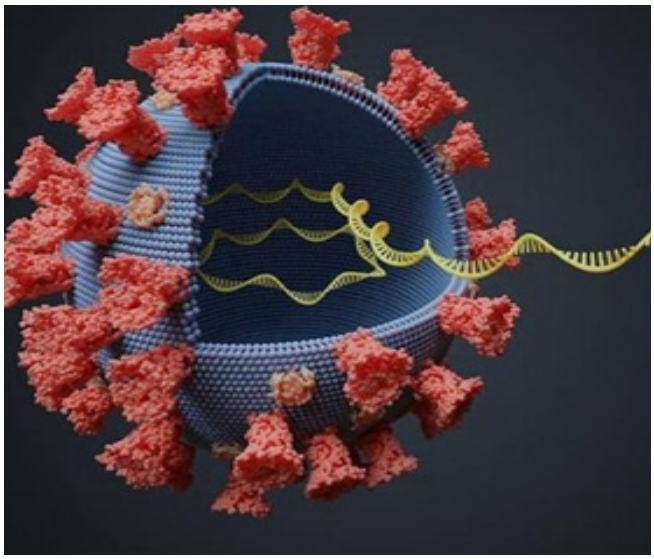

Fig. 4. Injectible vaccine to be replaced by a capsule.(After Koshy, J. 2021)

Types of Vaccines in development

Quite a good number of vaccines, nearly 180, are in the process of development to equip mankind for a fight against the deadly pandemic COVID-19 (WHO. 2020). A vivid description of these is available on WHO's website- https://www.who.int/publications/m/ item/draft-landscape-of-covid-19-candidate-vaccines. The platforms on which these vaccines are being manufactured in different labs worldover, have basically followed two approaches- 1. The Traditional one, like inactivated or live virus vaccines, platforms that have recently resulted in licensed vaccines (recombinant proteins, vectored vaccines) and 2. The platforms that have never been used for a licensed vaccine (RNA and DNA vaccines) (Krammer, 2020). In addition, the pre-clinical trials of certain protein vaccines have also started; in fact several S and RBD vaccines have entered into the clinical trials.

Replication inactive vectors

The adenovirus (AdV) based approaches that incorporated Replication inactive vectors dominated the scenario but modified vaccinia Ankara, human parainfluenza virus vectors, influenza virus, andeno-associated virus (AAV) and Sendai virus are used as well. The intramuscular delivery of these vectors enables their entry into vaccines' cells, followed by the response of the host immune system after the spike protein is expressed. Several advantages and disadvantages are obvious. The procedures for production need not necessarily be handling Live SARS-CoV-2. Notable experience has been gained by the scientists worldover to produce larger quantities of some of these vectors (an Ad26 prime MVA boost-based ebolavirus vaccine was recently licensed in the European Union) and they stimulate both B-cell and T-cell responses well. The impact of pre-existing immunity by the presence of vectors could partially neutralize these vectors. This being the disadvantage, has been overcome by using rarer vector types occurring in human beings which are derived from animal viruses or by viruses that are not potential immunity inducers by themselves. In addition, vector immunity can be problematic when prime-boost regimens are used which can be circumvented by priming with one vector and boosting with another vector. Several of these replication incompetent vector vaccines have undergon different stages of clinical development with ChAdOxnCoV-1947 (chimpanzee AdV), Janssen (AdV26) and Gamaleya Research Institute (Ad5/Ad26)50 as well as ReiThera (gorilla AdV).

Inactivated or Dead vaccines

Inactivated vaccines are produced by growing SARS-CoV-2 in cell culture, usually on Vero cells followed by chemical inactivation (Wang *et al.,* 2020, Gao *et al.,* 2020). The yield of these vaccines is the factor of virus productivity in the cell culture under the strict biosafety level 3 (BSL3) requirement. Examples are CoronaVac (initially called PiCoVacc), developed by Sinovac Biotech Ltd. in China as well as several other candidates developed in China, by Bharat Biotech in India and by the Research Institute for Biological Safety Problems in Kazakhstan.

The intramuscular administration of these vaccines are adjuvenated with alum or other adjuvants (Talon *et al.,* 2000; Wang *et al.,* 2020; Gao *et al.,* 2020). Under such a situation, not only S but in addition, the matrix, envelope and nucleoprotein are targeted by the immune responses due to the presence of whole virus in the human body's immune system. The Indian clinical trials of inactivated vaccine candidates, namely Covaxin has emerged successful.

Live attenuated vaccines

The immune response generated by natural infection are imitated by the genetically weakened viruses that have the potential for limited replication to produce live attenuated vaccines (Talon *et al*., 2000). The virus is permitted to adapt to the unfavourable conditions (e.g. growth at lower temperature, growth in non-human cells) or by rationally modifying it (e.g. by codon de-optimization or by deleting genes responsible for counteracting innate immune recognition).

An important advantage of these vaccines is that they can be given intranasally and induce mucosal immune responses which can protect the upper respiratory tract, the major entry portal of the virus. In addition, since the vaccine virus is replicating in the vaccinee, the immune response will likely target both structural and non-structural genes with antibodies and cellular immune responses (Wang *et al.,* 2020; Gao *et al.,* 2020). Of course, there are also disadvantages to these vaccines including safety concerns and the need to modify the virus which is time-consuming if done in the traditional way and technically challenging when reverse genetics is used. Only three live attenuated vaccines are currently in pre-clinical development including one that is attenuated by codon de-optimization in collaboration between Codagenix and Serum Institute of India.

Recombinant protein vaccines

The Recombinant protein vaccines comprise recombinant S vaccines, recombinant RBD vaccines and virus like particle (VLP) vaccines . These recombinant proteins can be expressed in different expression systems including insect cells, mammalian cells, yeast and plant (Zhang *et al.,* 2020; Talon *et al.,* 2000; Broadbent *et al.*, 2016). RBD-based vaccines can likely also be expressed in *E. coli*. Depending on the expression system yields and posttranslational modifications vary. The immune response triggered could be impacted by modifications like deletion of the polybasic cleavage site, inclusion of two (or more) stabilizing mutations inclusion of trimerization domains, particularly for recombinant spike, as well as the mode of purification (soluble protein versus membrane extraction). It is advantageous that without handling the virus, the vaccines could be generated. Additionally though, the license has been provided to some recombinant vaccines, like FluBlok for influenza. However, there are also disadvantages in S being relatively hard to express, that could possibly influence on the quantum produced as well as the doses quantified. RBD is easier to express. However, it is a relatively smaller protein when expressed on its own and while potent neutralizing antibodies bind to RBD, it lacks other neutralizing epitopes present on the full length spike.

DNA vaccines

DNA vaccines are based on plasmid DNA that can be produced in large scale in bacteria. Typically, these plasmids contain mammalian expression promotors and the S gene which is expressed in the vaccine upon delivery.

The high stability of plasmid DNA and the possibility of large scale production in *E. coli* are the advantageous aspects of these technologies. However, the DNA vaccines having expressed low immunogenicity, could be made more efficient by utilizing delivery devices to make them reach their destination. The need for such delivery devices, like electroporators, limits their use. Four different DNA vaccines have undergone different phases of clinical trials.

The huge advantage of these technologies is as well as. However, DNA vaccines often show low immunogenicity and have to be delivered via delivery devices to make them efficient. The need for such delivery devices, like electroporators, limits their use. Four different DNA vaccines have undergone clinical trials for the benefit of society.

RNA vaccines

A recent revolutionary development has been the generation of RNA vaccines to fight the corona virus outbreak. In an identical fashion to DNA vaccines, the delivery of genetic information is ensured by the antigen instead of its self expression by the antigen itself (Amanat *et al.* 2020). The expression in the vaccine's cells of the antigen then occurred. Two technologies exist: Either mRNA (with modifications) or a self-replicating RNA are used. The self-replicating RNA bears the capacity to amplify itself, while the mRNA required higher doses. Lipid nanoparticles (LNPs) are normally used to deliver RNA. Higher prospects of RNA vaccines have been ascertained in recent years, and several of them have been developed. A number of candidates have been incorporated in the promising pre-clinical results published (WHO, 2020; Chen *et al*., 2020; Vogel *et al.,* 2018). The vaccines of Pfizer and Moderna have finally emerged successful after Phase III trials.

Curevac and Arcturus have been in Phase I/II trials and are the candidates by Imperial College and the Chinese Liberation Army (Laczkó et al., 2020; Zhu et al., 2020). Advantages of the technology are that the vaccine can be produced completely *in vitro*. However, the technology is new and it is unclear which issues will be encountered for large scale production and the riddle of long term storage stability was resolved to assign 2-8° C for storage of Covaxin as well as Covishield; -18° C for storage of Sputnik V; -70 to -80° C for storage of Pfizer and Moderna. In addition, these are injected vaccines which are unlikely to induce strong mucosal immunity (Lu, 2020, Jackson, 2020 and Mulligan, 2020).

FastTrack procedure to expedite production of vaccine

As depicted in Figs. 5a,b. The pre-clinical processes/ toxicological studies normally consume 2-4yrs during formal vaccine development; followed by 1-2yrs during Phase I testing, 2yrs during Phase II, and Phase III another 2-3yrs. Thus a total of 5-7 years are required to complete Clinical Trials during normal development of a vaccine. After these stages being completed, 1-2yrs might further be consumed to cover Regulatory review by FDA, EMA etc., and finally large scale production and distribution begins. Therefore, in nut shell, a total of 15years or longer are expected before a fully characterized vaccine is released into open market (Figs. 5a,b).

On contrary to this, only a few month's period consumed on Pre-clinical/toxicology studies. Followed by before production of full fledged vaccine, followed by the overlapping clinical phases during trials, would drastically reduce the total time of vaccine production to 1.5years or 10 months. This precisely is the mechanism of FastTrack production method of vaccines applied for the production of Covaxin, Covishield and other vaccines in the fray.

The different aspects of the normal vaccine development as well as the FastTrack process have further been elaborated in Figs. 6a,b.

Molecular Epidemiology

The viral genome sequencing data generated by the RGSLs is analysed and sent to the National Centre for Disease Control (NCDC), Delhi for collation, integration and further necessary public health action through Integrated Disease Surveillance Program (IDSP).

The data is correlated and analysed to study the linkages between the genomic variants and epidemiological trends to understand super spreader events, outbreaks and strengthen public health interventions.

Clinical prospective

Linking this data with the IDSP epi data and patient's symptoms will allow us to better understand the viral infection dynamics, morbidity and mortality trends. Further, the data can be linked with host genomics, immunology, clinical outcomes and risk factors for a more comprehensive outlook. Knowledge generated through this vital research consortium will also assist in developing diagnostics and potential therapeutics and vaccines in the future.

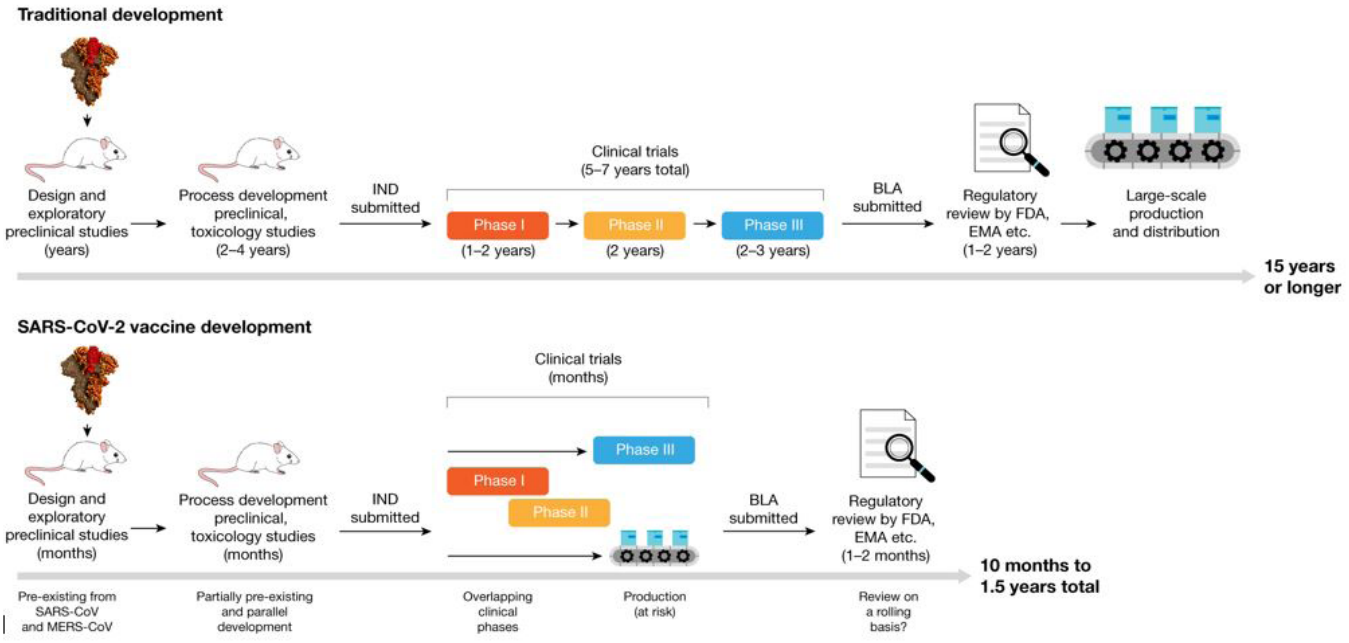

Fig. 5a. The duration of time normally required for the development of a vaccine. (after Krammer, 2020)

Fig. 5b. The duration of time normally required during Fast Track development of a vaccine. (after Krammer, 2020)

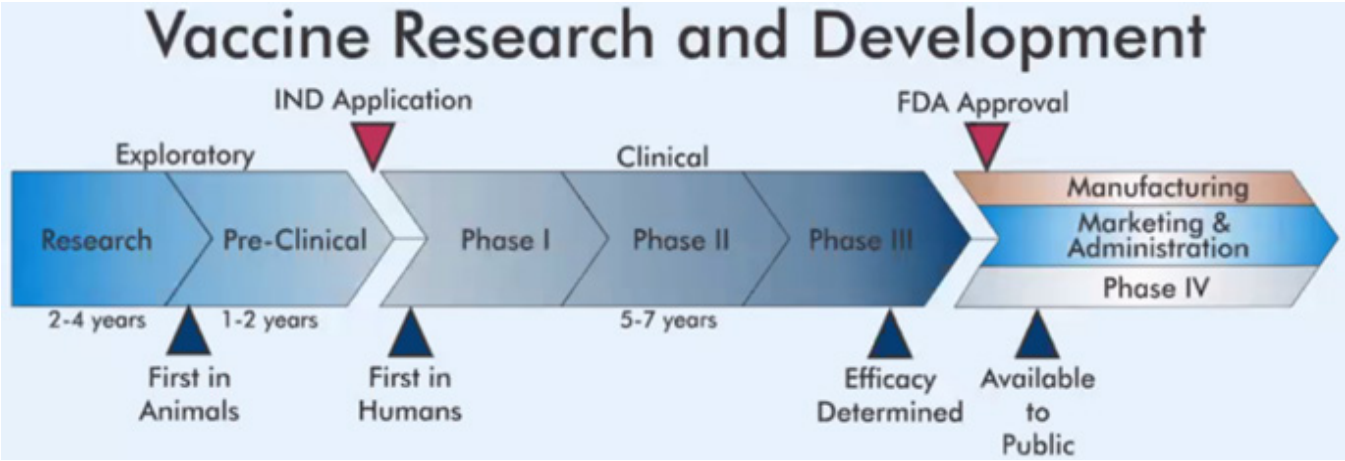

Fig. 6a. The expeditious research and development for Indian vaccines (Concept: after Krammer, 2020).

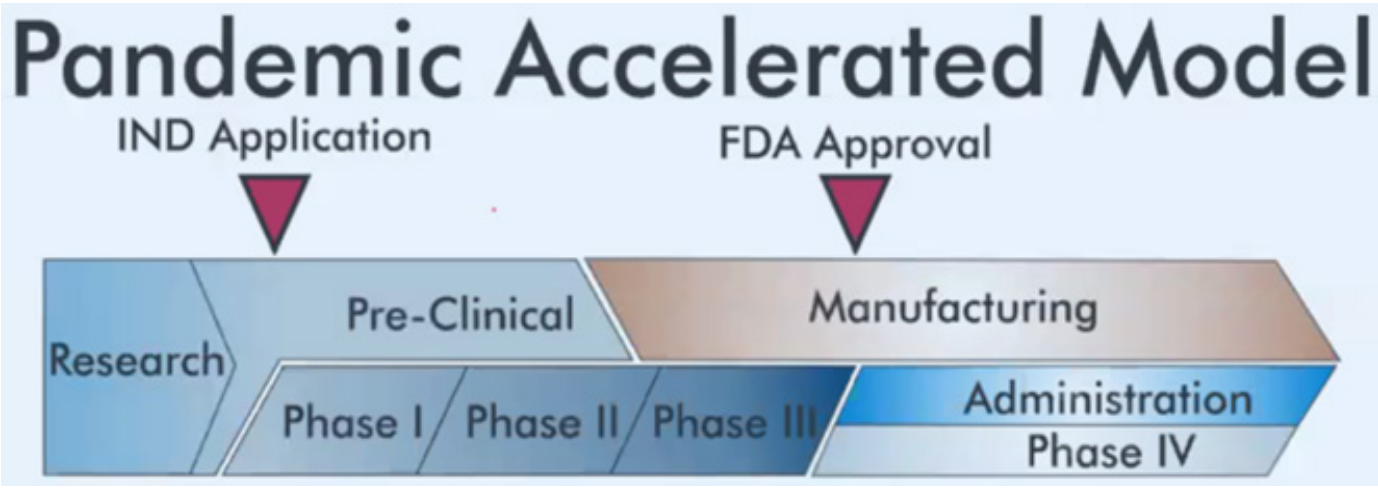

Fig. 6b. The acceleration of approval at par with the fattrack mode of development of vaccines in India (Concept: after Krammer, 2020).

Public Health Relevance

Overall aim To monitor the genomic variations in the SARS-CoV-2 for understanding the mutations/variants & their public health significance.

1. Disease scenario : Surges, rate of disease transmission, severity and fatalities.
2. Disease epidemiology: Morbidity & mortality in different age groups affected. Sex, geographic locations, present/past and future scenarios.
3. International impact: IHR obligations.
4. To understand relevance to testing, vaccination, immunity.

So far, 15,133 SARS-CoV-2 genomes have been sequenced from Covid-19 positive international travelers as well as from the community.

Implications

i. Overall better understanding of

1. Disease epidemiology. Time place, person and transmission. Disease trajectory in states.
2. Testing strategy
3. Clinical severity.

ii. State/District

1. Understanding of epidemiological scenarios.
2. Clinical picture of disease
3. Plan and strengthen preparedness.

iii. All individual level. Not relevant.

1. Testing.
2. Preventive measures remain the same.
3. Clinical management.

iv. Others:

Fig. 7. Brief depiction of symptoms due to the old strain of SARS-CoV-2.

1. National & International level.
2. Re-infection:
    1. Repeat infection
    2. Infection and vaccination: single dose, full coverage etc.

Manifestations of the older and newer strains of corona virus, SARS-CoV-2

The manifestation of the older strain of corona virus, SARS-CoV-2 exhibited fever, persistent cough as well as a loss of smell or taste in the infected patient (Fig. 7). However, the recently encountered newer strain showed altered symptoms of the disease *viz.*, aches and pains, conjunctivitis, sore throat, diarrhea, rashes on the skin, discoloration of fingers or toes and headache, after transformation of the older strain into the newer strain (Fig. 8).

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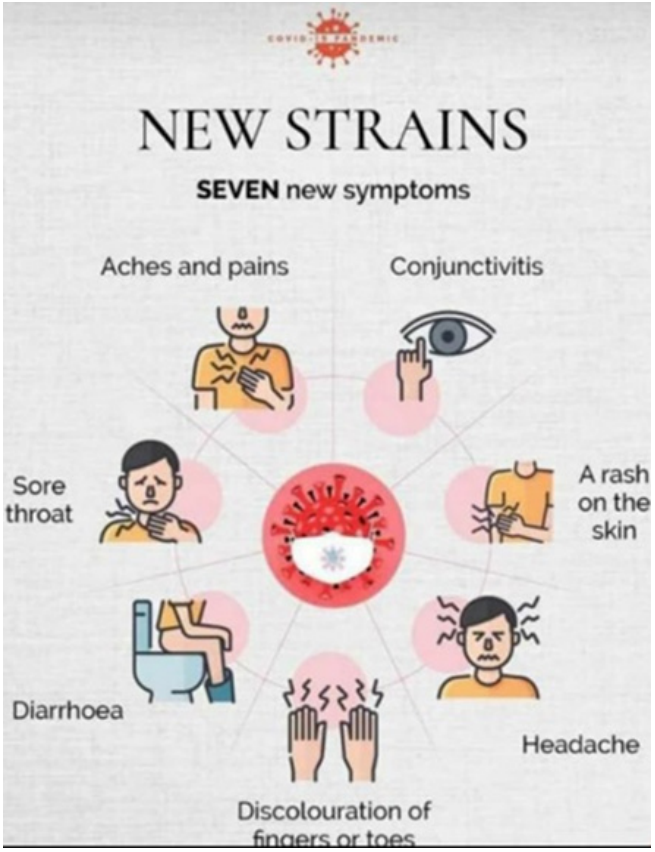

Fig. 8. Brief depiction of symptoms due to the new strain of SARS-CoV-2.

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