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hospital against medical advice. The outcome of secondary infections is depicted in Figure 1.

| Mortality due | Mortality due | Mortality due | Unknown | Recovered |
|---------------|---------------|---------------|---------|-----------|
| to blood stream infection | to respiratory infection | to fungal infection | | 52% |

52% Recovered
34% Unknown
6% Mortality due to fungal infection
3% Mortality due to blood stream infection
5% Mortality due to respiratory infection

Figure 1. Outcome of secondary infections.

Conclusion: Secondary infections play a vital role in increasing the morbidity and mortality in COVID-19 patients. The common isolates were Carbapenem Resistant *Klebsiella pneumonia* and Multidrug resistant *Acinetobacter baumannii*. Based on the time taken between COVID-19 positivity and development of secondary bacterial infection we can say that these pathogens could be nosocomial. Most of the bacterial isolates identified are multidrug resistant implying that empirical antimicrobial therapy might not be useful in such cases. Adhering to antimicrobial stewardship guidelines and strict infection control practices can help reduce the transmission such resistant pathogens in healthcare settings. The most common fungal isolate was *Rhizopus spp.* The primary reason for dissemination of fungal infection could be improper glycemic control and rampant use of corticosteroids and immunomodulators. Picking up the sentinel signs of mucormycosis early and employing multimodal therapy with antifungals and surgical debridement can play a vital role in reducing this fungal menace.

PCO-008
Identification of daclatasvir as a repurposed drug against Nsp15 of SARS-CoV2 (COVID-19) by using in silico approaches
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Background: In December 2019, SARS-Cov-2 epidemic was reported in Wuhan, China and then it spreads widely affects millions of people around the world. Nsp15 is one of the key members of EndoU family which perform several biological functions like RNA endonuclease activity which generate 2'-3' cyclic phosphodiester termini. In viruses, Nsp15 is conserved among nidoviruses and absent in other RNA viruses which makes it potential target for recent coronavirus outbreak.

Methods: In this study, we have used earlier studied Benzopurpurin B which has inhibition property against Nsp15 protein of SARS virus (0.2 μM). Next, we have developed structure-based pharmacophore model with the help of Benzopurpurin B and crystal structure of Nsp15 endonuclease NendoU from SARS-CoV-2 (6vww). For pharmacophore development, we have employed two different software, viz., Discovery Studio 4.0 and Ligandscout. The selected pharmacophore was used to screen FDA approved drugs from DrugBank Database. The hits retrieved were next subjected to molecular docking analysis followed by molecular dynamics studies.

Results: The best pharmacophore model A with 6 features (2 hydrogen bond acceptor, 2 hydrogen bond donor and 2 hydrophobic group, AADDHH) was selected based on highest selectivity score of 11.155. the validated hypo model 1 able to screen out 136 drugs out of 2454 FDA approved drugs from DrugBank Database. These drugs were further filtered out using molecular docking to remove any false-positive hits. Finally, 3 top hits were selected for MD simulation to confirm their binding stability.

Conclusion: Daclatasvir (DB09102), an antiviral approved drug was identified as possible candidate for designing the potent inhibitor against Nsp15 of SARS-CoV-2 virus, although further evaluation via wet lab is required to measure its efficacy.

PCO-010
Real-time PCR detects 4 rapid transmission variants of SARS-CoV-2
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Background: Currently in the world there are many variants of SARS-CoV-2, among which there are 4 variants: Alpha (B.1.1.7), Beta (B.1.351), Gamma variant (P.1), and Delta variant (B.1.617.2) has a faster transmission rate than the original strain by 82%, 161%, 50% and 198%, respectively. To detect the SARS-CoV-2 variants circulating in a certain endemic area, the method that the researchers are currently using is to sequence the entire genome
of the viruses detected in the samples. However, the sequencing method has the limitation that it cannot be applied in clinical laboratories.

**Aim of the study:** Design a test kit using multiplex real-time PCR that can be performed in diagnostic laboratories to detect 4 variants Alpha, Beta, Gamma and Delta and two mutations that help the virus to spread rapidly (D614G) and can escape the action of specific antibodies (E484 K).

**Material and method:** Primers and probes to detect Alpha, Beta, Gamma and Delta variants are designed based on the detection of specific mutations of these variants. The Alpha, Beta and Delta variants were detected based on ARMS Taqman real-time PCR (ARMS: Amplification Refractory Mutation System) with the principle that if a mutation is present, the Taqman probe will not be hydrolyzed and will not have an amplified signal, if there is no mutation the Taqman probe will be hydrolyzed and will have an amplified signal. The Gamma variant and the D614G mutations as well as the E484 K mutations were detected based on the SNP Taqman real-time PCR with the principle that each mutation would be detected by two Taqman probes with different reporters, FAM and HEX or TexasRED and CY5 and depending on the early or late of the fluorescent signal of these two Taqman probes, it can be concluded whether or not there is a mutation. The test kit is designed with three RT multiplex real-time PCR with multiplex A (MPL-A) to detect SARS-CoV-2 based on E gene using primers and Taqman probe (FAM) according to WHO design, variant Alpha (HEX) and the internal control is the RNaseP gene (CY5); MPL-B detects Delta variant (FAM), Beta variant (HEX), and Gamma variant (TexasRED/CY5); MPL-C detects D614G (FAM/HEX) and E484 K (TexasRED/CY5). The multiplex was prepared from AgPath-ID™ One-Step RT-PCR (ThermoFisher, USA). To check the primers and probes, the corresponding DNA sequences for the mutants were also designed as controls [+]. The test kit is then tested on samples that are the RNA extracts positive with SARS-CoV-2.

**Results:** Testing on [+ ] controls showed that the detection limit for the E gene and the Alpha variant was 10-5 fm/μl, the Delta variant was 10-5 fm/μl, and the Beta and Gamma variant was 10-7 fm/μl, the D614G and E484K mutations were 10-5 fm/μl. There was no crossing-detection of mutations or variants. Tested on RNA extracts that were positive with SARS-CoV-2, the results said that: In KCMC, the strain (1) taken in April 2020 is the wild type, while all strains (12) taken in June 2021 are Delta variants with additional mutations D614G and no mutations E484 K. In Quang Nam, the samples taken in June 2020 are both wild type (2) and have mutation D614G (3), while in June 2021 all strains were variants Alpha (4) and has the D614G mutation. The sample with the wild type, with the Delta variant, with the Alpha variant and the sample with only the D614G mutation were sequenced the whole S gene and the results were completely consistent with the real-time PCR results.

**Conclusion:** According to the laws of evolution, a rapidly spreading variant will gradually replace the original wild strain, and once community immunity to a variant is achieved, it may be susceptible to another variant and it can therefore replace the old one. Therefore, it is necessary to develop and set-up the multiplex real-time PCR test to detect 4 rapid transmission variants in diagnostic laboratories. With the collected results on the stock samples, we can conclude that at the beginning of the epidemic in Ho Chi Minh City, SARS-CoV-2 was still the original wild strain, but now it has been completely replaced by the Delta variant. In Quang Nam, the beginning of the epidemic was a wild strain but also circulating a strain with a D614G mutation, however the Alpha variant is currently circulating and has a D614G mutation. Particularly, the E484 K mutation has not appeared so far and this is an indication that SARS-CoV-2 has not yet been resistant to specific antibodies that recognize the receptor on the spike protein of the virus.

**Keywords:** SARS-CoV-2 variants, RT Multiplex real-time PCR.