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Journey of a Developing Nation's Microbiology Laboratory in COVID-19 Times: Challenges Faced

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ABSTRACT

The COVID-19 pandemic struck the resource-deprived developing nation like India as a lightning bolt in a clear blue sky and clinical microbiological laboratories had a critical role to play during this pandemic situation, not just in the diagnosis of the disease but also in surveillance. However, unlike most laboratories in the developed countries with the essential infrastructure and financial resources, the current situation posed enormous challenges for laboratories based in the developing world. This article is based on the experience from the clinical microbiology laboratory of a 967 bedded tertiary care hospital that provides comprehensive health and rehabilitative healthcare to the residents of North India drawn from the urban/semi-urban and rural areas. This review aims to highlight the challenges faced by a clinical microbiological laboratory in a developing country amid the COVID-19 crisis, changes implemented and the lessons learned. We have also added certain innovative and resource and manpower-saving techniques we used to increase the testing capacity and decrease the turn- around time of testing for our laboratory.

BACKGROUND

Coronaviruses (CoV) are a group of enveloped RNA viruses, ranging from 60 nm to 140 nm in diameter with a crown-like appearance, found most commonly in humans and birds [1]. Coronavirus has a total of seven strains which include HKU1, NL63, 229E, and OC43, SARS-CoV, MERS-CoV, and SARS-CoV-19 (COVID-19 the latest disease), out of which the first four present with mild respiratory symptoms whereas the other three may cause severe life-threatening diseases [1]. The history of the Coronavirus Disease 2019 (COVID-19) outbreak dates back to 31st December 2019, when pneumonia of unknown etiology was informed to the World Health Organization (WHO) initially detected in Wuhan, China. The outbreak was declared as Public Health Emergency of International Concern (PHEIC) on January 30, 2020, and was termed as a 'pandemic by WHO on 11th March 2020 [2]. It has continued to cause severe morbidity and mortality in most countries globally with catastrophic connotations [2]. In India, from 3 January 2020 to 15 June 2021, there have been 29,570,881 confirmed cases of COVID-19 with 377,031 deaths, reported to WHO. [3] As of 7 June 2021, a total of 238,840,635 vaccine doses have been administered. [3] The COVID-19 pandemic struck the resource-deprived developing nation like India as a lightning bolt in a clear blue sky [4]. Clinical microbiological laboratories always have a critical role to play during the pandemic situation, not just in the diagnosis of the disease but also in surveillance. However, unlike most laboratories in the developed countries with the essential infrastructure and financial resources, the current situation posed enormous challenges for laboratories based in the developing world.

The Indian Government along with the Ministry of Health and Family Welfare (MoHFW) and Indian Council of Medical Research (ICMR) worked intensely to minimize the number of cases by not just upgrading the testing facilities with multiple testing centers and testing kits but also by regularly updating the testing guidelines thus taking all necessary steps to combat the challenges and threat posed by this growing invisible pandemic war [5, 6]. Ours is a 967 bedded tertiary care hospital that provides a comprehensive health and rehabilitative healthcare to the residents of North India drawn from the urban/semi-urban and rural areas. This review aims to highlight the challenges faced by a clinical microbiological laboratory in a developing country amid the COVID-19 crisis, changes implemented and the lessons learned.

1.Initial challenges faced

1a) Establishing a molecular laboratory

The first suspected case for which SARS CoV-2 RT-PCR (reverse transcriptase-polymerase chain reaction) was requested at our hospital was a healthcare worker who had come in contact with a patient who returned from Singapore inMarch 2020. There was this initial scare and fright in the technical staff working under the Viral Research and Diagnostic Laboratory (VRDL) and the Postgraduate Junior Residents (PGJR) for collection of the sample but they did overcome their fears and took the first respiratory sample wearing Personal Protective Equipment (PPE) and all necessary standard and contact precautions [7]. This first sample was then sent to the National Institute of Virology (NIV), Pune which was the only testing laboratory for COVID-19 at that time in India [8]. Transport of a biohazard material requires triple packing which was of utmost importance in a SARS CoV-2 sample, considering the risk of transmission from fomites [9]. Samples collected after this was sent to All India Institute of Medical Sciences (AIIMS), New Delhi and later to the Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh as we did not have the required test kits for the COVID-19 RT-PCR testing at our institute. Finally, we could start testing at our institute from 27th March 2020.

The biggest challenge faced by us was the setting up of the testing laboratory and the coordination of sample collection for the same. What made things easier was the operational VRDL (Virus research and diagnostic laboratory), Medical College level laboratory in the department, started just a few months back. VRDL is a network of laboratories established by the Department of Health Research, Ministry of Health & Family Welfare, Government of India, as a part of the implementation of the Scheme “Establishment of Network of Laboratories for Managing Epidemics and Natural Calamities” to strengthen the infrastructure of viral diagnostics in India [10]. This initiative of creating the infrastructure for timely identification of viruses causing significant morbidity at the public health level, developing capacity for identification of novel and unknown viruses, emerging and re-emerging viral strains, and developing diagnostic kits, played a pivotal role in managing this COVID-19 pandemic in India. [8]

1b) Core Committee for COVID-19 management

A committee was made on the very first day which included the administrative head and officials and all the nodal officers from all the specialties. The committee members would meet every day the first thing in the morning and discuss the challenges faced the previous day and came out with solutions to handle the problems and prepare for forthcoming challenges too. Any emergency issues were dealt with, on the WhatsApp group of the members.

1c) Management of patients at the main laboratory

Our clinical microbiological laboratory of a tertiary care center serves both outpatients and inpatients. One major fear was the local spread of infection from un-screened/undetected patients and attendants visiting the facility for diagnostic workup and also for admission purposes [11]. A special counter was set up with appropriate safety protocol outside the molecular laboratory premises for screening using a standard form with necessary patient details for contact tracing if needed. Suspected COVID-19 cases were refrained from entering the laboratory premises and were diverted to the hospital's specially designated area for screening and management of such cases with the appropriate protocol. As COVID-19 is transmitted by droplets and close contacts, patients, staff and visitors to the laboratory had to wear mandatory masks and were provided with hand sanitizers at the counter and movement in common corridors was restricted. Social distancing in the premises was to be followed very strictly.

1d) Staff mental well-being measures

Even though we had a VRDL molecular laboratory but the number of trained staff to handle the molecular testing procedure was very less and we needed to train more technicians as quickly as possible to handle the abundant sample size. **The importance** of protecting healthcare workers (HCWs) from COVID-19 was a priority to maintain a safe and functioning healthcare system and to prevent it from collapsing. The risk of transmitting COVID-19 to family members was a source of stress for many. [12] Though there was a strict lockdown and implementation of social distancing in the entire nation to break the chain of transmission, still the frontline warriors of the laboratory kept toiling hard to provide early diagnosis to the patients to keep up the **"test treat track**" protocol of the ICMR [13]. It was very essential to keep the staff motivated at all times.

They were constantly encouraged by the seniors in the department and the administrative officials about the positive aspect of their work to create recognition. Furthermore, the management was reinforced to ensure easily accessible channels for staff feedback and concerns. COVID pandemic generated a lot of stress and anxiety in our laboratory personnel particularly in those with pre-existing mental health problems, with elderly or infants at home and in those with a chronic immunosuppressive disease like diabetes mellitus etc. It was essential for all laboratory personnel, seniors and junior staff, to remain connected throughout this crisis for technical, academic and personal reasons to ensure good and positive workplace environment.

1e) Internal surveillance measures

All healthcare workers serving in the molecular laboratory were strictly made to wear surgical masks and gloves as per the safety guidelines and regular audits were done to ensure compliance. Staff members who developed symptoms were asked to report immediately and were given medical consultation within the hospital staff clinic. This was re-assuring for all the workers and taken positively by all the staff. Additionally, refresher training on the use of PPE, hand hygiene practices, BMW guidelines was conducted regularly to reinforce good clinical practices (GCP) in them.

1f) Housekeeping activities

Housekeeping cleaning schedules were re-defined and revised and high exposure areas like the toilets, lifts, desks, tables were cleaned every 2-3 hours with 5% hypochlorite and alcohol-based sanitizers as appropriate. The premises were mopped three times a day with disinfectant. Discarded PPE and other clinical waste were collected in biohazard bags and disposed properly only after following proper BMW guidelines. [14]

1g) Continuous medical education (CME) via virtual platforms

An integral component of an academic institute is the propagation of knowledge to everyone including faculty, senior residents, post-graduate trainees, technicians and trainee technologists by lectures, case-based discussions, instrumentation modules for residents and journal clubs. To nurture the teaching and learning environment and continue the routine teaching-learning activities the teaching sessions were shifted to online teaching activities. Virtual learning environments were explored by the faculty and all live lectures and academic sessions were being conducted via Microsoft Teams for synchronous teaching with the

provision of recording for those who miss it. After getting familiar with Microsoft Teams, the section has been conducting all administrative and educational meetings on Microsoft Teams or even ZOOMS. The faculty was formally trained to operate this software by the information technology department and further utilized as trainers for residents and other staff members. These virtual platforms were highly efficient and effective as one could participate from their desk via their cell phones alongside the performance of other tasks assigned rather than scheduled events requiring their physical presence to reduce the exposure risk.

2. Sample collection, transport processing, and result dispatching

2a) Sample collection

According to the WHO guidelines [15], minimum of respiratory material should be collected. Upper respiratory specimens like nasopharyngeal (NP) and oropharyngeal (OP) swab or wash in ambulatory patients and/or lower respiratory specimens like sputum and/or endotracheal aspirate or Broncho-alveolar lavage in patients with more severe respiratory disease can be used for testing [16]. Since pooled nasopharyngeal swab and throat swab gave the highest sensitivity of 97% (95% CI 93–100), as compared to saliva (85%, 75–93) and lone nasal swabs (86%, 77–93) and throat swabs (68%, 35–94) [17][18], both NP and OP swabs were collected by dacron or polyester flocked swab and put in the properly labeled VTM vial to increase the viral load. The entire process from sample collection to transport was done by following biosafety precautions and using personal protective equipment (PPEs), in standard triple packaging and maintaining a cold chain. [15][16]

The Specimen Referral Form (SRF) format was provided by ICMR which was used initially [19] but later we came up with our own innovation to avoid handling of forms to prevent the fomite transmission of SARS CoV-2 [20]. The samples were collected by the experts of the area, Postgraduate junior residents (PGJRs) and Senior Residents (SRs) of the Otorhinolaryngology (ENT) Department wearing proper PPE and following biosafety precautions and the packaging of the sample, form filling was done by the PGJRs of Microbiology Department. We devised a format where all the essential details of the SRF were put in a tabulated format so that details of as many as 5 patients could be included on a single sheet thus decreasing the paper involved and risk of fomite transmission. A WhatsApp group was created where the photos of the detail sheet were sent which reached the laboratory personnel's on their mobiles. The detail sheets were discarded in the wards

themselves in yellow bags after sending the pictures on Whatsapp and not brought to the laboratory to decrease the risk of transmission. Also it acted as an effective medium for disseminating patient-related information to a wider working staff, thus allowing official agencies to reach a broad sector of the frontline workers rapidly and updating a wider audience in not just the patient details but also in circulating and updating the follow-up guidelines from the government agencies.

2b) Ensuring adequate supplies and inventory

The day the lockdown was declared i.e. 21st March, we had a very low stock of VTMs and swabs for sample collection and no dealers were able to provide us with VTMs as the transport had come to complete shutdown. We were in a state of panic as we could arrange for only some VTMs that a dealer had in stock at that time. ICMR came to the rescue by providing VTMs from the allotted depots for the same. Adequate supply of Personal Protective Equipment (PPE) as an essential requirement was ensured with the assistance of the purchasing department. The laboratory staff was counseled to practice judicious use of PPE and sanitizers. Inventory in charges was reinforced to re-visit their inventory requirements owing to the expected delays in shipment, to maintain the demand-supply chain.

2c) Testing for COVID-19 by RT-PCR

The most commonly used method for COVID-19 testing is based on Real-Time Polymerase Chain Reaction (RT-PCR) technique for identifying the genetic material from SARS CoV 2 [21]. This is one of the latest and sophisticated techniques of detecting genetic material. This method involves the reverse transcription of the genetic material of the virus, RNA to complementary DNA (cDNA), followed by amplification of targeted regions of the cDNA. Several serial amplification cycles are performed on real-time basis to identify these targets [22]. The technique sounds simple but it requires a lot of expertise and trained staff for performing the test and reporting it. We were already performing RT PCR-based tests for the diagnosis of swine flu (H1N1) for the past four years, so we had a running molecular laboratory in place but the challenge was a trained staff for performing a test for this high number of samples. The technical staff was sent to PGIMER, Chandigarh, which is also the regional VRDL lab for our medical college-level lab, for training.

The procedure of testing involves three main steps:

1. Decontamination – This is the first step where the procedure is carried out in a Biosafety Cabinet, wearing PPE and handling the sample with all precautions. The sample becomes noninfective at this step.

2. Extraction of RNA – The genetic material, which is the RNA in the case of SARS CoV-2, is extracted at this step.

3. Mastermix preparation and amplification – The master mix contains nuclease-free, forward and reverse primers, a fluorophore-quencher probe, and a reaction mix (consisting of reverse transcriptase, polymerase, magnesium, nucleotides, and additives).

The master mix and extracted RNA are loaded into a PCR thermocycler in a 48 or 96 well plate, and the incubation temperatures are set to run the assay. During rRT-PCR, the fluorophore-quencher probe is cleaved, generating a fluorescent signal. The fluorescent signal is detected by the thermocycler, and the amplification progress is recorded in real-time. [23]

We had two RTPCR machines in our molecular laboratory at that time, one was the existing machine and the other was provided and installed by ICMR under VRDL. The RT PCR kits, extraction kits and VTMs were provided by ICMR and we performed mock runs for 3 days before declaring our hospital as a COVID-19 testing center. We analyzed and resolved the various problems that we faced in those runs and finally, when we were confident, we started testing at our laboratory on 27th March 2020. There were days when the whole run would go invalid and we had to repeat the whole process again but we troubleshot the problems and kept our laboratory work.

As mentioned earlier, the RTPCR kits and extraction kits were provided by ICMR, different RT PCR kit and extraction kit with different protocols were received every time. This was again a challenge as this required standardizing the new kit with known positive and negative samples before putting the kits to use for testing as a part of quality control. Another problem with the RT PCR kits was, different kits target different genes for amplification. Every time a new kit was received, it took us time to understand it and report accordingly. The various RTPCR kits that were used at our institute are given below (Table 1).

Table No. 1: Shows various kits and their targets used at our Laboratory

Sr. No.	Name	Type	Targeted genes				
			E gene	Orf1b	RdRp	N gene	S gene
1	ICMR-NIV Pune[24][25]	Different wells	√	√	√		
2	BGI Genomics, China [26]	Single well, Multiplex	√	√			
3	Tru PCR [27]	Single well, Multiplex	√			√	
4	Diag Suren COV 19 Detection assay (Multiplex Taqman)[28]	Single well, Multiplex	√	√			
5	Q Line [29]	Single well, Multiplex		√		√	

2d) Pooling of samples

Since the positivity rate was <2 %, in May we started pooling of samples for testing to save the resources and to increase the numbers of tests conducted by the laboratory and to maintain the turnaround time (TAT) [30]. We pooled a maximum of 5 samples only in one pool after carefully considering the clinical history, history of contact with a positive family member, category of the patient from SRF form which was least likely to come positive. As the positivity rate started to rise(>2-5%), we stopped pooling and went ahead with individual testing as deconvoluted testing of each pool delayed the report and led to the wastage of reagents. [30]

2e) Reporting of RT PCR

The first kit that was used was ICMR-NIV Pune [24][25]. This was a two-step process kit, screening and confirmatory. The first RT PCR run was performed for E gene for all samples which is the screening run. The ones that came positive for E gene were then subjected to the confirmatory run targeting Orf1b and RdRp genes. If both or any one of the two came positive the patient was reported as positive [24][25]. The other kits were based on multiplex

PCR and targeted all the genes in the same well. This reduced the turnaround time and dispatching of reports earlier as the result was generated after a single run only. E gene is a gene shared by all beta coronaviruses and the presence of this, only signifies the probability of COVID-19 infection while all other four genes are specific to SARS CoV-2 and their presence is confirmatory for diagnosis of COVID-19. [31]

The result of RT PCR is generated on the screen in the form of S-shaped sigmiod graphs which can be observed in real-time as the target gene amplifies. The target starts multiplying exponentially and starts to rise in log phase and once the building material in form of dNTPs is exhausted, it plateaus.[32] Besides the target genes for the SARS CoV-2 there is another gene that is also targeted, RNase P. The Internal RNAse P control provides a nucleic acid extraction procedural control and second negative control. Some kits have an Internal Control (IC) provided with the kits which is targeted for amplification acting as the control for extraction and amplification steps. The validity of a run is dependent on the positive and negative controls. A run is only valid if the negative control shows no amplification and positive control shows amplification, failing which the run is invalid and has to be repeated.[33]

2 f) Dispatching of Reports

This step was time-consuming and effortful. The reports were sent electronically via email. All the reports were scanned and named separately and then mailed so every patient could have access to their separate reports. Later we decided to prepare a single report of a single run in a tabulated form which lessened the burden.

2 g) RT PCR App and Uploading of Reports on ICMR portal

All the reports generated were uploaded on the ICMR portal from the very first day of testing with all the essential information including categories. ICMR had created categories for different patients depending on symptoms, travel, contact, healthcare worker, and others. ICMR kept updating these categories from time to time. Later ICMR came up with the RT PCR App which was linked with the ICMR portal. All the information was put in the RT PCR app at the time of sample collection which generated a SRF ID. The SRF Id when put in the ICMR portal transferred all the information automatically and only the result was added later [19].

CONCLUSION

Most health care organizations and the general public worldwide were not prepared to face the emerging threat of the COVID-19 pandemic. The agenda and correct outline for such a crisis never existed in the books or journals especially for resource-constrained setups in a developing world like ours. While we still cannot predict what is in store for us in near future or when the next major pandemic will bolt us, thorough preparedness and lessons from past experiences will act as a guiding light for future generations. Crisis response should be part of the regular laboratory training. If feasible, prior standard operating procedures (SOPs) addressing the challenges the lab can face during the pandemic should be in place for immediate action. The VRDL Scheme by DHR-ICMR was a big support to all the testing laboratories all over India at this time. It has brought molecular RTPCR testing to the smallest of the labs working in COVID-19 testing and strengthening the healthcare system.

In the present situation of an extensive spread of this rapacious disease not just in the country but also in the entire globe and amongst the high chances of contracting COVID-19, it is inexorable and imperative that the clinical microbiological laboratories take substantial measures to plan for the smooth working of diagnostic services for better health outcomes and also to keep the safety and interests of its valuable employees. COVID times have been a difficult time, yet a time of great learning for all of us. We were pushed to our edge to perform and give our best and rise above our fears. We, in the future must be prepared to adapt to the constantly evolving phases and emergent clinical scenarios during any pandemic with a proactive approach. This pandemic gave us a different world, with a different set of rules; for the work environment, and social norms, both at home and the workplace. The earlier we adapt and embrace changes, the easier the challenges will seem.

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