"Microbiological Advancements in the Post-COVID Era"

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Introduction

Covid 19 pandemic had consumed the world since its onset in Dec 2019 when first case was detected in Wuhan China.The pandemic poseds a litmus test for the whole health care system of the country.Due to the unprecedented demand for testing with over whelming number of samples,RTPCR labs, which were earlier used only for research purposes had to be set up on a war footing in all medical colleges and private hospitals.Not only this,the out break augmented the urgency to develop a number of robust rapid and portable devices and instruments. Kit based tests were designed for a rapid turn around time, as opposed to conventional PCR. Virology labs all over India got a face lift and they were armoured with state of the art facilities. Apart from this, another aspect which gained paramount importance was infection control. This aspect which was overlooked all these years, garnered all the attention and infection control practices, starting from simple hand hygiene, utility of PPE, and biomedical waste management, were all taken seriously and came into daily practice. Lastly, this pandemic gave us a first hand experience of dealing with a pandemic and also an insight about how to go about an out break management. On the other hand its also worth while mentioning that this pandemic had siphoned all the attention of clinicians and scientisits, that other major concerns like MDR and TB and even chronic ailments like diabetes and hypertension have been neglected. This editorial is a peek into the influence of COVID 19 pandemic on different aspects of Microbiology.

Utility of Molecular techniques in diagnostics:

Molecular diagnostics are a blend of molecular techniques , human genetics and knowledge of medicine. Techniques like PCR, deals with amplification and detection of DNA fragments using short nucleotide segments called Primers. In RTPCR, RNA are converted to Cdna sequences by reverse transcriptase followed by amplification of the newly synthesized Cdnaby standard PCR procedures. Other than RTPCR, Molecular diagnostic techniques used in some of the clinical laboratory are FISH, microarrays, MALDI-TOF, and nucleotide sequencing. FISH (Flourescence in situ hybridization)works on the principle of detecting the required sequence by hybridizing them with specifically designed fluorescent probes and detecting the signal under a fluorescent microscope. It is used to detect pathogens, and also to diagnose specific features in nucleic acids found in tumors, cancers, amniotic fluids, etc.Microarry involves binding of millions of known nucleic acid fragments on a silicon or glass substrate (chip). Then the chip is bathed with labelled DNA or RNA from an unknown sample. Target DNA fragments along with complementary sequences bind to DNA probes. Target DNA can be identified by their fluorescence emission by passing a laser beam. Microarray has been used for genotyping, forensic analysis, evaluating germ line mutation etc.

Molecular diagnostics in Post Covid era:

Loop-Mediated Isothermal Amplification (LAMP) : Due to the dire need of tests with a short turn around time with increased sensitivity and specificity, new methods were launched.Loop-mediated isothermal amplification (LAMP) is an alternative to the tedious PCR technique. The fact that this procedure utilizes a single temperature, i.e., 60–65 °C, for amplification, and also doesn't need a thermocycler makes this method much easier and economical compared to PCR.. It can amplify the DNA in 25–35 min using polymerase byusing high DNA-strand replacement activity using 4–8 specific primers [1]

ILAMP has been widely adopted for the detection of many pathogens, such asmalaria, Salmonella, Influenza virus, dengue, chikungunya and Zika virus. LAMP can be easily combined with the RT reaction (i.e., RT-LAMP), directly detecting target RNA without a separate RT step. In this way, the total reaction time can be greatly shortened.Similarly, multiplex RT-LAMP combined with lateral flow biosensors has also been developed with high sensitivity and specificity LAMP can also be used for detection of SNPs for other diseases. It acts as a powerful tool for point of care diagnostics.

Biosensors : Biosensors is an economical robust less time consuming sensing and analytical device which can provide continuous and real time detection. It can measure and transduce a physical signal(electrical, mechanical, thermal, optical) produced from a biological change.These biorecognition elements could be whole cells, DNA, RNA, and products of immune response like antibodies pathogens, proteins, glucose, anti-microbial peptides produced in response to foreign evading bacterial or viral peptides in organisms.Nano biosensors are antibody based or DNA based which allow optical , electrochemical or field effect transistor based transduction.

Bio receptorcombines with the analyte and a transducer, to convert the captured virus in the sample into a detectable signal; Eventually electronic systems amplify and express the signal for quantitative detection.2,3. Many research studies have developed novel biosensors for detecting SARS-CoV-2, such as field-effect transistors (FET)on biorelectric recognition assay, the LOD dose of which is 1 fg/mL, and the detection time is only 3 min. Additionally, this portable biosensor can be controlled via smartphone or tablet.4Biosensors have been used for detection of Tuberculosis, Staphylococcus, and infections by gram negative organisms.

CRISPR-Based Diagnostics—SHERLOCK and DETECTR : One of the most remarkable innovation in the field of molecular diagnostics is CRISPR-Based Diagnostics. Clustered regularly interspaced short palindromic repeats (CRISPR) is a part of the natural immune system of microbes for protection against foreign material by recognizing them and eliminating them via CRISPR-associated endonuclease Cas enzymes .

DETECTR and SHERLOCK are 2 analogous diagnostic systems which have been designed to detect RNA and DNA respectively. First viral or bacterial sequences, from the clinical samples are amplified using recombinase polymerase amplification. The resulting DNA or RNA is mixed with a CRISPR/Cas system which can identify and also cleave the sequence of interest. The Cas endonuclease for RNA (DETECTR) Cas 12 A and for DNA it is Cas 13 a (sherlock). The Cas endonuclease, is so designed that it starts to cleave indiscriminately only after it is activated by binding to its target . When a quenched reporter sequence is introduced, Cas12a or Cas13a will cleave the reporter sequence, resulting in a fluorescent signal.The intensity of light directly depends on the quantity of the amplified sample. However, if the target sequence is not present in the solution, Cas12a and Cas13a will not be activated and the reporter sequence remains quenched. SHERLOCK and DETECTR are so sensitive that they are capable of single copy viral detection. Moreover, the enzymatic reactions work at 37 oC, and do not require expensive thermal cyclers. They are also rapid, taking only 1–2 hours from start to finish. This technique showed 90% sensitivity and 100% specificity. This technique is used to detect SNPs, Zika virus, pathogenic bacteria, and dengue virus [5] . AIOD-CRISPR and FELUDA are other modified versions of techniques using CRISPR in diagnosis[6]

Aptamer-Based Diagnostics :Aptamers are artificially made small oligonucleotide or peptide sequences that target specific DNA or RNA of interest.They are small biomolecules ranging from about 20 to 60 nucleotides and by binding specifically to the target sites, mimic antibodies. Aptamers bind with a high affinity and specificity to a wide range of targets, such as bacteria, viruses, whole live cells, proteins, peptides, small molecules and metal ions.The small size of this biomolecule makes it a suitable candidate for a stable target in diagnostic techniques. These random nucleic acid sequences are incubated with a target molecule. Unbound molecules get separated from bound ones. Eluted nucleic acid are then amplified by PCR . Eluted nucleic acid are amplified by PCR and serve as an enriched library for the next cycle. SELEX (Systematic Evolution of

Ligands by Exponential Enrichment) technology is a method used to develop aptamers. A sandwich assay is developed to detect Leishmania major proteins, where in these Aptamers are coupled with magnetic beads and fluorescent molecules 7Other applications are for detection of Malarial parasite, salmonella, influenzas etc.According to FDA reports of April 2022, 294 advancements in molecular tests, 90 in antigen depending tests, and 34 serological tests have been approved[8].

Heightened awareness and technological advancement in infection control measures in Post COVID times:

Post covid, infection control practices have grabbed importance in health care. Everyone has realized that simple measures like hand hygiene, wearing mask ,gloves etc, goes a long way in controlling infections, whether it is hospital or community acquired. Awareness about PPE, donning and doffing procedures, and proper BMW disposal was created among not only health care personnel but also general public. Infection control officer and ICN had to play a major role in monitoring the adherence of HCW to the norms of infection control. As monitoring all the healthcare workers round the clock posed difficulties, electronic monitoring systems were sought and developed.

Some recent developments in IP technologies include electronic hand hygiene monitoring systems, antimicrobial textiles, ultraviolet C (UV-C) devices, The electronic medical records can be used as a support for decision making and also for predictive analysis to prevent health care associated infections. Direct observation is regarded as the gold standard for hand hygiene monitoring. However, direct observation is costly in terms of staff time and resources and may lead to biased adherence rates—for example, because of the influence of complex social interactions between the observer and the observed person

3 categories of technological innovations for hand hygiene monitoring purposes based on their respective functions: (1) behaviour monitoring systems, (2) data management tools, and (3) technique improvement systems. Studies have found that technological innovation sustained the effect of improved hand hygiene compliance at a rate that was 1.5times greater in the postintervention phase—lasting for 63 days—compared with the preintervention baseline.[9]

Electronic hand hygiene monitoring systems (EHHMSs) offer hospitals large data sets of hand hygiene (HH) compliance among health care workers (HCWs). Post covid, usage of mask, hand sanitizers and hand washing practices have definitely increased and this will help to prevent other diseases and infections as well.

Powered Air-Purifying Respirators (PAPRs)run on a battery operated motor which helps in sucking the particles in the gas through a filter and cartridges. Then this air is delivered under positive pressure to a face piece. The positive pressure reduces leakage of possible contaminants. PAPRs are known to provide more protection compared to NP5 masks.They have an assigned protection factor (APF) of 25 . In addition, they eliminate the fit problem and can be worn with eyewear[10].

There have been a significant change in the last decade in the health care setting which has resulted in an expanded IPC program scope of responsibilities[11].

There is a very limited data on impact of COVID 19 on health care associated infections. It is also widely variable. Cole et alreported an increase in health care workers' compliance with infection control precautions, leading to a decrease in multidrug-resistant organism infections in their health care facility during the pandemic.[12]

According to a brief report, reported to the National Healthcare Safety Network: United States,in a children’s hospital CLABSI standardized infection ratios decreased when comparing 2016–2019 (−26%, 95% CI [−31%, −20%]), and increased from 2019 to 2022 (18%, 95% CI [9%, 26%]).13This stresses on the fact that though there has been an awareness about IPC practices, consistent and resilient practice of the same is required. However, previous studies found that COVID-19 negatively impacted HAI's and led to multidrug-resistant organism in-hospital outbreaks.

During the pandemic there was a concern about the raising number of hospital acquired infection,but inspite of this there was a reduction in the number of respiratory and health care associated Clostridium difficile infections.[14]RRight from the start of the pandemic, Strict adherence to PPE and sustained reinforcement on Patient isolation, and reduction of patient visits could be a reason for this decline.

Awareness about zoonotic emerging infections: According to the WHO, zoonotic diseases are infections transmitted from nonhuman species to humans and can be bacterial, fungal, viral, or parasitic.Bats harbor more than 60 species of viruses like rabies virus and other Lyssavirus (Family Rhabdoviridae), Hendra and NiV (Paramyxoviridae), Marburg and Ebola viruses (Filoviridae), MERS-CoV and SARS-CoV (Coronaviridae) to name a few[15]. A complicated network between the host agent and the environment has offered many avenues for the transmission of diseases causing pathogens to humans. Though these human spillovers can lead to small outbreaks, if adequate measures are taken it can be halted. If not, this may become a concern for affecting a large world population in pandemics. The recent unforeseen COVID pandemic is a clear example to reinforce the fact that a zoonotic virus believed to emerge from a live market in Wuhan province in China can cause a deadly zoonotic disease among mankind. In recent history, a Nipah virus outbreak was seen in Kozhikode, Kerala, in September 2021, where a 12-year-old boy presenting with acute encephalitis tested positive for NiV and died from the infection.

One of the recent out break that occurred in UK on 7th may 2022 was Monkey pox. This created raising fears that the virus may spread and cause an outbreak, particularly amid the continuing COVID-19 pandemic. Cases increased after this by 10 th June 2022, from 43 nations over 1500 cases were identified. After that, the cases multiplied; by 10 June 2022, over 1500 cases from 43 nations, including those in Europe and North America, were reported. India reported the first case in South Asia on 14 July 2022. So far there have been 10 confirmed cases including one, which is a locally transmitted case, with no history of travel.

Strategies adopted by India during the COVID-19 pandemic that can help in future zoonotic disease pandemic control: India has worked towards aspects like training the health care workers, strengthening the health care system, providing education and training to all level of medical professionals so that they are confident of working in areas like screening camps, isolation wards, hospital wards, ICU.

Also the government has provided PPE, aids like oxygen plants, effective indigenous vaccines etc. Also digital India has helped people to gain information at all levels including treatment and counselling.Public awareness and education with International cooperation: mass communication;preparedness, and resource planning; providing vaccines not only to the home country but increasing production to provide support to other countries.Also the government has provided PPE, aids like oxygen plants, effective indigenous vaccines etc. Also digital India has helped people to gain information at all levels including treatment and counselling.Public awareness and education with International cooperation: mass communication;preparedness, and resource planning; providing vaccines not only to the home country but increasing production to provide support to other countries.

To achieve the best health outcomes for humans, animals, plants, and our environment, (India's One Health approach proposition)there is a need for communication, coordination, and collaboration.16 A promptly deployed outbreak containment response and the strict biosafety practices followed during the COVID-19 pandemic in Kerala possibly helped to prevent further transmission of NIV and to restrict the outbreak[17].

Conclusion:

The COVID 19 pandemic has taught mankind ample life lessons. In the field of Microbiology, the pressing need for rapid, accurate molecular diagnostic tests,paved way for newer ground breaking innovations like CRISPER, Point of care biosensor technology, Aptamers etc which have very promising role in the field of diagnostics. Biosensors can become the most effective alternative to immune assay method and also used for verifying the pathogens of infectious diseases. CRISPER based therapies for viral and bacterial infections, CRISPER vaccines have also been developed. After COVID, RTPCR has become a house hold diagnostic method, which was not so in the earlier days. In the post COVID times the NABL accredited labs can be utilized for other viral diagnostics and can be also be stepped up with next generation sequencing.Post COVID, people have become aware of the concept of infection prevention,

be it hand hygiene or wearing mask. Even in hospital settings IPC measures are laid out meticulously, but adherence rates have to be improved.The evolving technology in infection prevention can increase the patient adherence rates and also make the analysis easier. Future studies are required to explore the integration of infection prevention technologies into everyday practice. These innovations should focus on the validation of current technologies, particularly focusing on patient-centred outcomes such as HAI risk reduction. In addition, for a better understanding of the potential impact of these technologies on patient safety , cost-benefit analyses are also required.Over all, because of the pandemicit hasbeen possible todelve deeper into the field of virology, understand the equation between man nature and its inhabitants. It's a lesson learnt in a hard way, not to disturb the equilibrium of nature.

References:

1.CarpenterM.A, Geletkanycz, M.A, SandersW.G. Upper echelons research revisited: Antecedents, elements, and consequences of top management team composition. J. Manag. 2004, 30, 749–778.

2.Khan M. Z. H, Hasan M. R, Hossain S.I, Ahommed M.S, Daizy M, Biosens. Bioelectron. 2020, 166, 112431.

3.Mavrikou S, Moschopoulou G, Tsekouras V, Kintzios S, Sensors 2020, 20, 3121.

4.Alamri, A.M.; Alkhilaiwi, F.A.; Ullah Khan, N. Era of Molecular Diagnostics Techniques before and after the COVID-19 Pandemic. Curr. Issues Mol. Biol. 2022, 44, 4769–4789. https://doi.org/10.3390/cimb44100325

5.Ding, X.; Yin, K.; Li, Z.; Liu, C. All-in-One dual CRISPR-cas12a (AIOD-CRISPR) assay: A case for rapid, ultrasensitive and visual detection of novel coronavirus SARS-CoV-2 and HIV virus. bioRxiv 2020

6.Broughton, J.; Deng, X.; Yu, G.; Fasching, C.; Servellita, V.; Singh, J.; Zorn, K. CRISPR–Cas12-based detection of SARS-CoV-2. Nat. Biotechnol. 2020, 38, 870–874.

7.Bruno J. G., Richarte A. M., Phillips T., Savage A. A., Sivils J. C., Greis A., et al.. (2014). Development of a Fluorescent Enzyme-Linked DNA Aptamer-Magnetic Bead Sandwich Assay and Portable Fluorometer for Sensitive and Rapid Leishmania Detection in Sandflies. J. Fluoresc 24, 267–277. 10.1007/s10895-013-1315-6

8.FDA Updates ;2021 Available online: https://www.cdc.gov/cliac/docs/april-2022/3_fda-update.pdf

9.Radhakrishna K, Waghmare A, Ekstrand M, Raj T, Selvam S, Sreerama SM, et. al.: Real-time feedback for improving compliance to hand sanitization among healthcare workers in an open layout ICU using radiofrequency identification. J Med Syst 2015; 39: pp. 68.

10.Center of Disease and Control Hospital Respiratory Protection Program Toolkit NIOSH. [(accessed on 6 October 2021)];2015 Availableonline: https://www.cdc.gov/niosh/docs/2015-117/pdfs/2015-117.pdf?id=10.26616/NIOSHPUB2015117

11.Bryant KA, Harris AD, Gould CV, et al. Necessary infrastructure of infection prevention and healthcare epidemiology programs: a review. Infect Control Hosp Epidemiol. 2016;37:371–380.

12.Cole J, Barnard E. The impact of the COVID-19 pandemic on healthcare acquired infections with multidrug resistant organisms. Am J Infect Control. 2020:5726.

13.Christopher P , Lucy F, Prachi P Margaret D Jonathan E, Ronda S, Cochran, DK. A Review of Pediatric Central Line-Associated Bloodstream Infections Reported to the National Healthcare Safety Network: United States, 2016–2022 Journal of the Pediatric Infectious Diseases Society 2023;12(9):519–21

14.Ponce-Alonso M, Sáez de la Fuente J, Rincón-Carlavilla A, et al. Impact of the coronavirus disease 2019 (COVID-19) pandemic on nosocomial Clostridioides difficile infection. Infect Control Hosp Epidemiol. 2020:1–5.

15.Sudeep AB, Yadav PD, Gokhale MD, et al.. Detection of Nipah virus in Pteropusmedius in 2019 outbreak from Ernakulam district, Kerala, India. BMC Infect Dis 2021;21:162.

16.Aggarwal D, Ramachandran A. One health approach to address zoonotic diseases. Indian J Community Med 2020;45(suppl 1):S6–S8.

17.Yadav PD, Sahay RR, Balakrishnan A, et al.. Nipah virus outbreak in Kerala State, India amidst of COVID-19 pandemic. Front Public Health 2022;10:818545.