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The COVID-19 pandemics and the relevance of biosafety facilities for metagenomics surveillance, structured disease prevention and control

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic represents an enormous challenge to all countries, regardless of their development status. The manipulation of its etiologic agent SARS-CoV-2 requires a biosafety containment level 3 laboratories (BSL-3) to understand virus biology and in vivo pathogenesis as well as the translation of new knowledge into the preclinical development of vaccines and antivirals. As such, BSL-3 facilities should be considered an integral part of any public health response to emerging infectious disease prevention, control and management. Differently from BSL-2, BSL-3 units vary considerably along the range from industrialized to the least developed countries. Innovative Developing Countries (IDCs) such as Brazil, which excelled at controlling the 2015–2017 Zika epidemic, had to face a serious flaw in its disease control and prevention structure: the scarcity and uneven geographic distribution of its BSL-3 facilities, including those for preclinical animal experimentation.

1. Biosafety level requirements during epidemics

The preexisting healthcare infrastructure and research networks in a country are important assets to mounting an effective response against an emerging health threat. In poorer nations, where these components are less developed, the response to an epidemic has been primarily driven by outside experts who can often be severely constrained by local customs and societal norms [1]. These difficulties become more apparent when the manipulation of the underlying pathogenic agent requires higher biosafety levels for its containment.

A comparison of the responses in two countries to two viral epidemics of different biosafety levels can expose important considerations for future events. The emergence and spread of the Ebola virus, which requires containment in biosafety level (BSL)-4 facilities, occurred in some of the poorest countries of West Africa where no appropriate facilities existed to deal with such agent. The response, therefore, had to be led and organized from abroad [2]. In contrast, the response to the Zika virus was coordinated internally within the affected countries. The virus emerged in 2015 as a BSL-2 pathogen and spread rapidly throughout Latin America, which is home to a number of Innovative Developing Countries (IDCs) including Brazil [3]. The previous decades of scientific investments for research on other arboviruses, at both the federal and state levels of the IDCs, provided the necessary infrastructure for national researchers and institutions to deliver the science needed to develop an effective public health response [1,4].

The formidable threat of the ongoing pandemic of the coronavirus disease 2019 (COVID-19) has exposed the numerous challenges to combat emerging pathogens for not only poor countries, but the whole global community. We call attention to the disruptive challenge posed by the requirement for BSL-3 level facilities to conduct fundamental research on the causative agent, Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2). It has represented a major, critical bottleneck in the fight against the impact of COVID-19. Even in countries and institutions where such facilities exist, their high demand to perform studies on SARS-CoV-2 has become nearly impossible to satisfy. For example, based on pubmed.gov entries of published articles on coronaviruses, in one year 4-times more studies were done than since 1949. For animal experimentation, appropriate BSL-3 platforms are even more rare. In Brazil, for instance, the laboratories that excelled at generating relevant and important knowledge during epidemics of Zika, Dengue and Chikungunya have had to face long waiting lines to access BSL-3 facilities in their own or collaborating institutions [5,6] (preprints: [7–11]). Ultimately, this has had the effect of concentrating the ability to advance the preclinical development of vaccines and antivirals to the richest countries.

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2. Moving towards epidemics prediction and metagenomic surveillance

Fortunately, the two highly pathogenic coronaviruses that emerged before SARS-CoV-2, SARS-CoV and Middle East Respiratory Syndrome coronavirus (MERS-CoV), were controlled before they could spread. In contrast, the infection characteristics of SARS-CoV-2 in combination with increased travel patterns allowed it to spread worldwide quickly. Once again, immunologically naive human populations were exposed to a viral spillover [12]. The world was completely unprepared, from a health perspective to social and economic perspectives, to deal with what would become the most serious sanitary threat of present times. COVID-19 shined a glaring light onto an old dilemma: spending on disease prediction/prevention [13–15] or focusing on surveillance [16]? This never-ending discussion needs to be resolved based on the clear evidence of the potential economic toll caused by epidemics, which should convince everyone irrespective of their fiscal philosophy that investing in both approaches is not only a good health policy but also sound economics.

The emergence of SARS-CoV-2 in China is a good example of prediction: it could be detected and characterized in record time due to previous work on bat coronavirus [17] which has continued uninterruptedly as a part of the PREDICT funding programme [18] and the Global Virome Project [13]. An example of the need to improve surveillance comes from our demonstration that Zika and Chikungunya circulated during a year in Rio de Janeiro before being detected [19,20]. This observation points to a much-needed change in disease prevention and control: the need to invest in syndromic, metagenomic surveillance and not only on the diagnostics of specific diseases. The complete sequencing of suspected COVID-19 clinical samples has shown the usefulness of this approach as it has detected additional concomitant viral genomes that could possibly be involved in the clinical response of a patient (unpublished).

3. Central coordination and best practices are essential in every country

Many industrialized countries have succeeded in organizing, and funding, centers for disease control and prevention (US CDC, China CDC, European CDC). A major, on-going effort aims to establish a CDC in Africa, but similar initiatives are absent or immature in most developing countries. In Brazil, disease surveillance relies on a national system of public health laboratories (*Sistema Nacional de Laboratórios de Saúde Pública*, SISLAB) that was launched in 2004 and today includes twelve BSL-3 Units to handle infected cells and samples together with a network of centralized state laboratories (*Laboratórios Centrais de Saúde Pública*, LACENs) that are BSL-2 laboratories. To date, Brazil does not have a BSL-4 facility.

The BSL-3 units are certified by the Brazilian Ministry of Health (BRA-MoH) and are heterogeneously distributed throughout Brazil, a country nearly the size of a continent. Although the information is not explicitly disclosed by the BRA-MoH, there are fewer BSL-3 units in the Central-Western region of Brazil than in the Southeast region. Although good practices and well-trained personnel could handle complex endemic pathogens at a BLS-2 level, access to BSL-3 level facilities are critical for rapid responses to undefined epidemic and pandemic situations. In other words, it is tangible that this non-uniform coverage could in influence and shape public health responses. Indeed, the outbreaks of yellow fever virus (YFV) on the coasts of the Brazilian Northeast and Southeast in the summers of 2016/2017 and 2017/2018 were preceded by the circulation of the YFV in the Central-Western Brazil. Appropriate local infrastructure to rapidly identify and isolate complex pathogens, such as wild-type YFV, could have prevented or reduced the risk virus spread within Brazil and to other countries. Current network could be strengthened by establishing other federally funded BSL-3 laboratories within the system of Federal Universities to expand the capacity to respond to sanitary emergences.

In addition to qualified laboratories, BSL-3 animal facilities are another absolute requirement to perform the research necessary to generate new knowledge on highly pathogenic (re)emerging infectious disease. The development of interventions also strictly relies on animal models to fulfill the requirements of pre-clinical development of antivirals and vaccines. Many industrialized countries give high priority to these platforms, realizing that their investments have a potential to not only deliver needed health solutions, but also can provide a range of economic gains from start-ups to licensing new technologies. This potential for economic development benefits could help justify the costs of building and operating a BSL-3 animal facility, which has limited its available in developing countries. For instance, Brazil has no BSL-3 animal facility under the coordination of the BRA-MoH. This has led to a tough lesson from the COVID-19 pandemic as there is limited access in developing countries to critical lifesaving supplies due to their dependence on imported medicines, which can be related to an absence of the necessary infrastructure for “in-house development”. An example is the antiviral remdesivir that was developed by an American company and is mostly unavailable since its promising pre-clinical results led to the US government purchasing almost the entire global supply of this drug.

Innovative Developing Countries would be more independent and could be able to respond to future epidemics if biosafety laboratories, particularly BSL-3 units, were integrated into a coordinated system that allows for the execution of the most complete scientific response. This would pave the way for the rapid preclinical development of vaccines and antivirals to emerging threats. This subject becomes of higher interest when we take into consideration that in tropical countries, many of the disease outbreaks may not capture the attention of pharmaceutical industry due to the low financial return. The Chikungunya virus is a good example: after almost a decade since its re-emergence in the developing world, no vaccine or specific treatment has been approved. In Brazil, scientists were only able to complete studies on drug repurposing for Chikungunya when the virus was downgraded to a BSL-2 pathogen, which was actually a response to the fact that its circulation had become endemic. It is unlikely that any of the highly pathogenic aerosol-generating viruses, such as Influenza A(H5N1) virus and SARS-CoV, SARS-CoV-2, MERS-CoV and Ebola virus, will have their biosafety level downgraded – reinforcing the necessity of BSL-3 facilities. The downgrading of the biosafety level of a pathogen is often unequivocal evidence of its autochthonous circulation.

The initial risk classification of a given pathogen and its revision is critical to integrate BSL-3 facilities into the public health response to an unfolding epidemic/pandemic. For example, when the Zika virus started to circulate in the Americas, the original perception of it being a self-limited disease, along with a previous history of endemic circulation of flaviviruses, made this virus a BSL-2 pathogen. Conversely, Zika virus was contrasted by the observation that a viral infection in pregnant women increased the incidence of microcephaly in newborns. Thus, in countries where Flaviviruses are not endemic, the vector is exotic or the population is immunologically naïve to Zika virus, higher biosafety requirement is prudent. In fact, the causative relationship between Zika virus and teratogenesis highlight that Koch's postulates are still contemporary. Thus, the causative relationship between a microorganism and disease should be established, on the bases of laboratory experimentation, which may require high biosafety level facilities.

Over the past decades, genome sequencing has become much more accessible and the tools to perform metagenomic- and metatranscriptomic-based surveillance more feasible. In the future, newly identified genomes will need to be associated with emerging disease. Normally, finding the nucleic acid of a microbe in a clinical sample is not sufficient to implicate it in disease. For diseases that are minimally reproduced in animal models or complex culture systems, isolated microorganism must be tested for their ability to cause disease in BSL-3 facilities as an integral part of public health systems. If a pathogen is fastidious and cannot be cultured, researchers may entail to identify epidemiologically linked individuals with a similar course of illness, within a certain period of time, as an alternative to satisfy Koch's postulates. The necessity to verify whether or not an emerging pathogen can be cultured stipulates the necessity to use biocontainment at a level of 3 or above and is even more critical in scenarios short to fulfill Koch's postulates.

Finally, the COVID-19 pandemics reinforces the necessity for both cellular and animal BSL-3 laboratories as a fundamental part of the public health

response to quickly identify potentially harmful microorganisms and to develop medical interventions.

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Conflict of interest statement

The authors declare that there are no conflicts of interest. Given his role as Editorial Board Member, Carlos Medicis Morel had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to the editor William J. Liu.

Author contributions

Thiago Moreno L. Souza: Conceptualization, Writing - Review & Editing, Funding acquisition. **Carlos Medicis Morel**: Conceptualization, Writing - Original Draft, Funding acquisition.

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