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The proteomics contribution to the counter-bioterrorism toolbox in the post-COVID-19 era

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1. The B threat from the CBRNE acronym and the black swan theory

The CBRNE acronym – for Chemical, Biological, Radiological, Nuclear, and high-yield Explosive weapons [1] – was proposed to designate the different types of threats counter-terrorism responders could be faced with. These unconventional weapons have the capacity to create devastating casualties among soldiers and civilians, and significant disruption of societal services, while also destroying the very cohesion of States. Rapid and efficient detection, and appropriate counter-measures, are essential if we are to mitigate these threats. Indeed, the consequences of an attack can only be significantly reduced by mounting an immediate and optimal response, which must be planned in advance. Unfortunately, the current world context of local and international conflicts, but also the trends in evolution of societies and human behavior, represent fertile ground for the development of higher-level risks. Among the C threats, priorities include biological toxins such as ricin and abrin, alongside chemical nerve agents and cyanide derivatives. The B threat corresponds to bacteria and viruses, living agents that can cause high mortality, are easily propagated, and are relatively resistant to classical antimicrobials or antivirals. The spore-forming bacterium *Bacillus anthracis*, responsible for acute infectious anthrax; the variola virus from the Orthopoxvirus genus, causing smallpox; and the Ebola Filovirus, which induces hemorrhagic fever, are just some examples of the most awkward known biological agents (see <https://emergency.cdc.gov/agent/agentlist-category.asp> for an updated list of high-priority agents by the US Centers for Disease Control and Prevention). The Black Swan Theory formalized by Taleb [2] in the statistics field considers a rare, hard-to-predict event with such a disproportionate impact that it could significantly re-route human history. Compared to CRNE threats, the B threat could be considered to fall within the Black Swan Theory because of the potential for extensive amplification of some living agents and the disastrous consequences that a wide release scenario with a more infectious, pernicious variant would entail.

2. CBRNE plans must be overhauled in a post-Covid-19 era

Humanity has dealt with a large number of plagues which caused untold deaths and dark times. A century ago, in 1918–1919, the so-

called Spanish flu caused the deaths of over fifty million people. This pandemic was triggered by the H1N1 influenza virus, but secondary infections by virulent pathogens, such as the bacterium *Streptococcus pneumoniae*, added to the heavy toll. Since December 2019, we have been dealing with the COVID-19 pandemic, caused by the SARS-CoV-2 betacoronavirus, member of the sarbecovirus lineage [3]. Like with influenza, the lung damage caused by the SARS-CoV-2 virus creates a favorable terrain for secondary infections, increasing the number of casualties. SARS-CoV-2 infection in humans may appear atypical, as its symptoms and long-term consequences differ considerably between patients. Most surprisingly, many infected people remain asymptomatic, although some suffer pulmonary lesions, and this has certainly contributed to the extensive spread of the virus [4]. **Figure 1** shows how the virus spread rapidly worldwide, triggering successive waves of infection in Asia – where it emerged in Wuhan, China, in December 2019 [5] – followed by Europe, and later the Middle East, North America, South America, Oceania, finally reaching Africa over the first quarter of 2020. **Figure 1** also shows how quickly variants emerged during the pandemic. These mutations can be easily monitored by whole-genome sequencing to trace the virus back to its source and establish the spread of each sub-strain. Noteworthy, these mutations may disturb the detection of the virus if they affect sequences or surrounding sequences corresponding to one of the primers used in qPCR detection or antigens recognized by antibodies used in immunodiagnosics. They could also impact the design and development of vaccine.

In addition to the terrible number of deaths and the dramatic number of people affected, the effects of the COVID-19 pandemic on modern human civilization have already been impressive, with unprecedented lockdown measures, drastic social changes, and extensive world-ranging economic crises. Even the most optimistic among us must admit that further unimaginable consequences are surely to come.

A large variety of coronaviruses exist in bats and other animals [3], and among them, some may be more fatal and harmful than SARS-CoV-2. While it is of interest to sample new lineages and variants to enhance scientific knowledge and develop appropriate counter-measures, these isolated and amplified strains also represent new dangers. SARS-CoV-2 infected material, which is currently widely available, could be stored with malicious intent for use at a later date to advance a particular cause. Generating

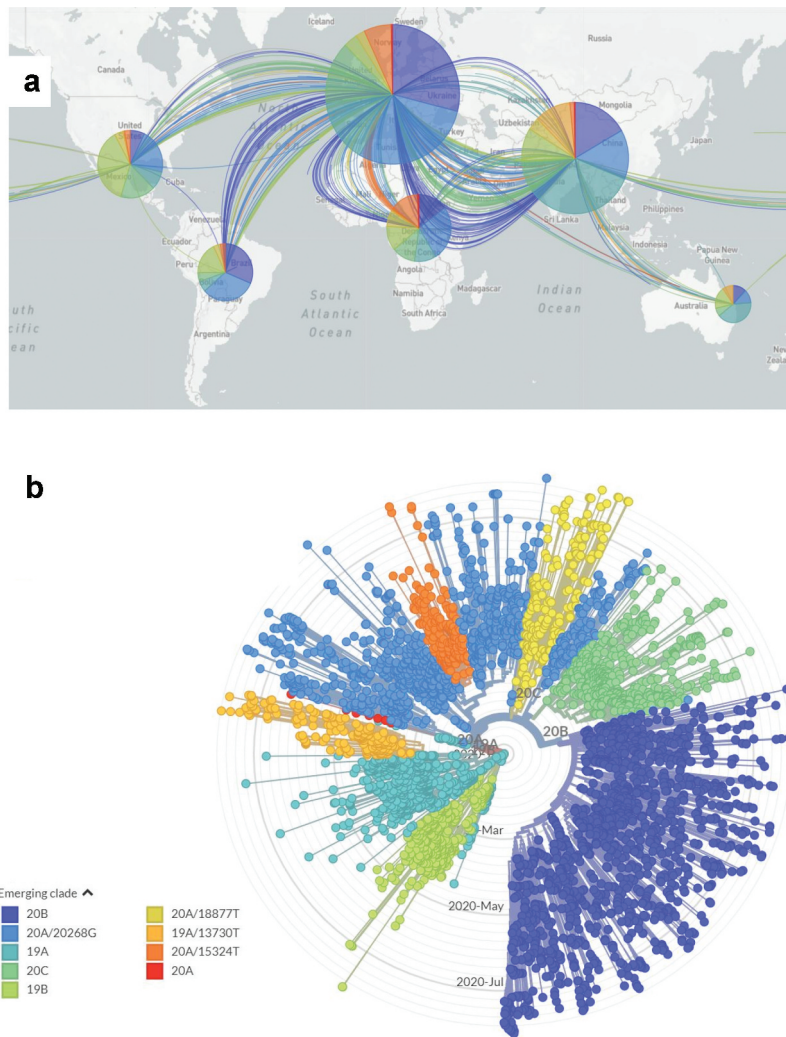


Figure 1. Spread of SARS-CoV-2 worldwide over the first half of 2020. The figure was created with the nextstrain interface (<https://nextstrain.org/>) using GISAID genomic epidemiology data on SARS-CoV-2 (<https://www.gisaid.org/>). Panel A shows how SARS-CoV-2 lineages, established from the ~75 000 sequences available, spread across continents. The radius scales with the number of genome sequences from each continent and the color of sectors and continent connections reflects distinct SARS-CoV-2 lineages. Panel B shows the different SARS-CoV-2 lineages and the time-scale for their emergence.

new synthetic variants of virus or even chimeric forms is nowadays an additional credible threat. Thus, in this post-COVID-19 era more than ever, coronaviruses and other harmful biological agents should be considered very high-priority in counter-terrorism circles. Consequently, current CBRNE plans should be thoroughly re-assessed to significantly enhance and improve tools and protective measures [6].

3. New opportunities for proteomics to accelerate pathogen detection

Proteins are discriminant taxonomical biomarkers that can be detected and quantified by mass spectrometry for rapid identification of potential threats. Whole-cell matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry has been implemented in clinical laboratories for routine microbiological diagnostic applications [7]. This approach relies on a mass fingerprint corresponding to the most abundant, small basic proteins, as illustrated in Figure 2. This approach requires

minimal sample preparation, but can only be applied to pure samples, typically obtained after overnight growth on agar. Indeed, the main reason this method is not the gold standard for use for CBRNE monitoring is that, in this field, detection methodologies must work on all sample types – medical or environmental. In addition, methods should not be perturbed by confounding effects, and ideally they should be quick. An alternative to whole-cell MALDI-TOF is a targeted proteomics approach, such as selected reaction monitoring (SRM), multiple reaction monitoring (MRM), or parallel reaction monitoring (PRM), which can detect specific representative peptides for the pathogen sought, the latter two methodologies allowing interesting multiplexing capabilities. Targeted proteomics method can be rapidly developed for any pathogen, can handle difficult matrices, and requires no pathogen-specific reagents, as demonstrated recently for SARS-CoV-2 [8–10]. Moreover, proteomics has shown its potential to provide faster detection than many alternative methods [9], but further developments are needed to miniaturize the sample preparation and mass spectrometry steps. In the CBRNE field, several reviews have high-

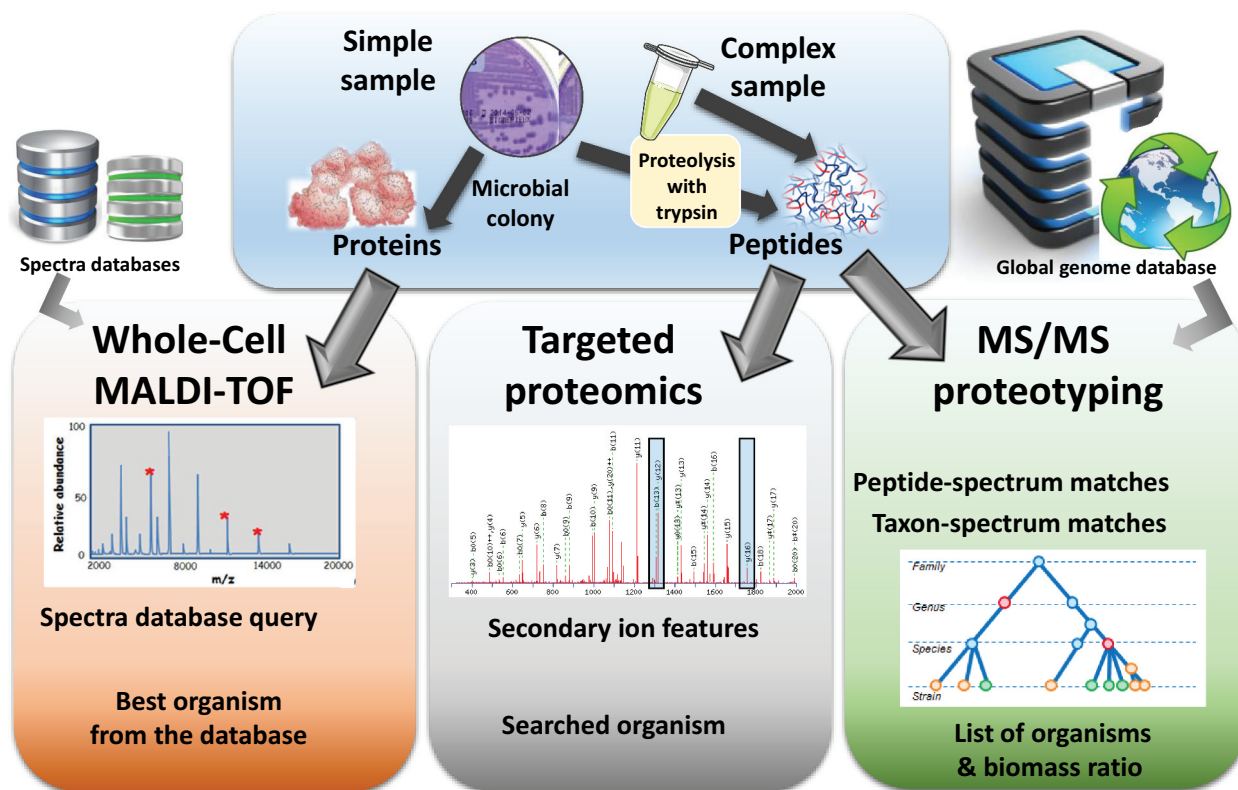


Figure 2. Strategies for proteomics-based detection of pathogens. Schematic representation of whole-cell MALDI-TOF, targeted proteomics, and tandem mass spectrometry proteotyping by shotgun proteomics.

highlighted the power of the targeted proteomics approaches [11], and many outstanding experimental studies deserve specific mention [12,13]. However, targeted proteomics approaches are not currently easy to extensively multiplex. More recently, other approaches relying on shotgun proteomics have emerged. Tandem mass spectrometry proteotyping relies on the without a priori identification of tens to thousands of taxon-discriminant peptides that can reveal the identity of the species present in a sample [14]. Figure 2 shows how taxonomic information can be derived from the peptides identified. Several bioinformatics programs have been proposed to handle the proteomics data garnered, and to produce lists of the agents present based on their discriminant peptides [15–17]. A recent modification, phyloproteomics, proposed the use of discriminant and non-discriminant peptides to avoid inflation of annotated genome data which tends to limit the list of available discriminant peptides. Phyloproteomics is based on mathematical modeling of the number of peptides shared between organisms [18] and was shown to be amenable to high-throughput applications [19] and would be compatible with mixtures of microorganisms. Interestingly, it not only provides taxonomical identification of the pathogens, but also sheds light on their molecular resistance to drug compounds. The current limitations of tandem mass spectrometry approaches are their sensitivity and throughput, but a wealth of opportunities for further improvements to proteotyping methods and the treatment of bioinformatics data exists, and should give rise to interesting developments in the near future.

4. Improved characterization of pathogens, their reservoirs, the mechanisms they use to cause infections, and novel therapies

Numerous proteomics strategies have been developed to study how pathogens function, in particular how they infect their hosts. Several studies of *B. anthracis* illustrate such approaches, including comparisons of strains with different virulence levels [20]. Their results revealed potential targets for the development of new drugs. Similarly, a multi-omics approach was used to decipher the virulence mechanisms of *Yersinia pestis*, the bacterium causing plague [21]. New research directions should now be developed to better understand how pathogens escape from the immune system, how mutations occur and are selected during host passaging, and to improve our knowledge on pathogen reservoirs. Next-generation proteomics should play a significant role in this ground-breaking research.

The emergence of multiple antibiotic resistance capacities in several pathogens is of major concern, and proteomics could also play a pivotal role in advancing our understanding of this process and how resistance spreads, while also contributing to the search for alternative drugs [22]. The power of proteomics could be harnessed to stratify patients, as demonstrated recently with Covid-19 infections [23], to identify biomarkers of exposure (to B or C threats), or to better understand the efficacy of vaccines and accelerate their development, as illustrated for the Lassa virus, an Arena virus endemic to parts of Western Africa [24].

5. Concluding remarks

A better knowledge of how bacterial pathogens and viruses – our age-old enemies – propagate is key to counteracting possible CBRNE threats. Next-generation proteomics methods can contribute to many aspects of basic research and development with potential for applications in diagnosis, prognosis, countermeasures, and therapies, together with genomics and other omics technologies. Thus, proteomics could play a determinant role in quick on-site diagnosis of disease, but only if manufacturers of high-resolution tandem mass spectrometers miniaturize their instruments and decrease costs. Such approaches could lead to the development of instruments similar to those used to detect explosives in airports, providing precise answers in minutes. Evidently, other technologies should be improved and benchmarked in parallel to continuously improve the counter-bioterrorism toolbox. The Covid-19 pandemic has, naturally, attracted huge attention to coronavirus-related research, and proteomics has proved to be quite successful in this field, demonstrating its capacity to rapidly gather new knowledge on a novel emerging pathogen [25]. However, the enormous impact demonstrated for this type of novel threat should make us aware of the need for preventive actions. Studies of other pathogens, especially those relevant to the CBRNE field and newly emerging lethal pathogens, should not be discontinued, but rather reinforced. Finally, international collaborations between researchers, technology providers, first responders, and governmental CBRNE services is needed more than ever to tackle the increasing CBRNE risks.

6. Expert opinion

Biological agents, or live pathogens, could represent unconventional weapons. Luckily, CBRNE counter-terrorism experts can rely on an extended toolbox of next-generation technologies to detect them and minimize their impact. However, the Black Swan Theory, which considers an extremely improbable event which would have a disproportionately large impact, may apply to biological threats. This scenario is possible because some living organisms have an amazing capacity for amplification, and propensity to spread, while also having drastic effects. The etiological agent responsible for the COVID-19 pandemic, SARS-CoV-2 virus, has shown how pernicious and damaging sarbecoviruses can be. In the post-COVID-19 era, we must re-assess biological risks without compromise, taking into account the three grey swan coronavirus known outbreaks and the most recent societal changes. Proteomics has important roles to play in improving and accelerating detection of pathogens and their potential resistance to current treatments, determining their source as quickly as possible, stratifying patients to improve therapeutic interventions, investigating new drug or vaccine therapies, characterizing pathogen reservoirs, and improving predictive epidemiological models. It thus constitutes an essential element in the counter-terrorism toolbox to deal with biological threats as developed here.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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