Towards Total Biothreat Preparedness: Expanded Surveillance, Joint Monitoring, Pooled Resources And The Genomic Option.

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Abstract

The current challenge in health monitoring is the extended spectrum of agents which emerge as human pathogens due to the deterioration of health, massive population movements which result in reshuffling the microbioma in receiving countries. Thus, new microbiota have to be included in monitoring and surveillance schemes of both civilian and military/security applications. The adoption of military developed hardware and procedures to civil application may save considerable funds and time and allow a unified, biospheric surveillance comprising plant, animal and human pathogens since they break through the species' or kingdoms' barriers much easier than thought. Fast turnover serodiagnostics and DNA diagnostics, developed for military use, have the potential to produce *ad hoc* new identification assays on common, adaptable platforms. This approach contrasts the costly, high-expertise diagnostic services, in order to counter a global threat of unknown kinetics but disastrous dynamics.
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1. Introduction

The international prospect of health, despite significant scientific and therapeutic advances, currently inspires decreasing levels of optimism [1]. The unavailability, in terms of accessibility and affordability of health products and services for an ever increasing proportion of the population within first world states due to cost, as accessibility is a very cost-dependent issue and affordability follows suit IF accessibility is achieved [2], diminishes the actual healthcare without really reducing its costs as expensive tests and substances replace previous, massively available inexpensive ones. The deterioration of health and immune standards for even larger proportions of an aging population due to questionable habits and increased life expectancy is followed by an unprecedented increase in emergence of pathogens: a multitude of actually emerging (new or modified/mutated/ evolved pathogens with new qualities and niches), locally emerging

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(pathogens appearing in different localities/ environments than usually detected, mostly travel & migration infections) or specifically-biologically emerging (finding new breeds of hosts, especially among the immunodeficient host) pathogens increase their incidence and combined with the aforementioned other factors, collectively compose a gloomy picture [3].

In many respects the deteriorating healthcare/humanitarian picture, with massive failures of infrastructures due to natural or man-made disasters, starts to resemble decades-old projected situations of military healthcare, especially of bioterrorism and biowarfare aspects. During the 90s the most important biosecurity threats were the offensive vaccination (as a delivery method) and reshuffled/genetic engineered, chimeric or *de novo* built pathogens, along with rare, combined/chimeric or engineered toxins (as main threat agents) [4]. On the latter issue, the main problems were the high levels of uncertainty caused by the lack of knowledge on the effector agents and the ability of the threat(s) to evolve and combine, thus dodging both proactive and reactive measures. The problem was clearly similar in many respects to current and projected non-military related health status, and far more so compared to then-current and projected forecasts.

Despite methodological breakthroughs such as MALDI-TOF [5], metagenomics [6] and high-output sequencing, also known as Next Generation Sequencing -NGS [7], for infection control there are two methods that have been, were and remain suitable: antibody-based detection and Polymerase Chain Reaction (PCR)-based detection and identification [8]-both, obviously highly dependent on database availability, integrity and accessibility within a demanding technical/temporal-spatial framework of specifications.

The former method is sensitive enough, robust in field conditions and easy to apply even without mechanical instrumentation. Antibodies fixed in strips permit energy-independent assays, a feature invaluable in many failed or 3rd world states and in battle/crisis [9]. But the genetic, protein and organism engineering advancements allow the prediction and subsequent deletion or alteration of epitopes without a change in functionality, especially in effectors were the epitope is unrelated or loosely related to the effector moiety/ies. As most engineered pathogens are likely to have inherent epitope concealment or alteration provisions, and given the time needed for new antibodies to be designed, selected and produced which may be expedited to 2 months or even less, but not any further [10], fast-pacing crises were overcoming such detection measures.

On the other hand, DNA analysis is less easy and user-friendly as it requires more steps, but much more robust, since both whole organisms and single effector moieties depend on DNA sequences to function (the former) or to be produced at any cellular level (the latter), with the possible exception of prions and virions. Genetic engineering and alternate coding (based on the inherent degradation of the genetic code) can be used to fool such detection systems, but they are costly, difficult and laborious. Random priming, real-time protocols and multi-locus approaches can overcome such countermeasures expeditiously. The problem with DNA and nucleic acids detection in general is that toxins, a well-known health issue and a bio-weapon *par excellence* [11] cannot be detected at all if used in a cell-independent mode, i. e. as a (bio)chemical weapon, thus necessitating for a dual-platform (nucleic acid amplification and seroimmunological).

2. The Joint Approach

The "Joint Approach" is a more conventional and less impressive term for what is called "total biothreat preparedness". in both cases the essence is to unify resources and he scope of the agents under scrutiny, instead of segregating them to human, animal and plant pathogens or to even narrower threat classification. this approach has two main pillars: the strategy, comprising temporal/spatial considerations, priorities, threat estimations and the means available to implement it- personnel and equipment/instrumentation, plus the ever necessary infrastructures (positioning/buildings, utilities, protection). The infrastructure and support part being totally a matter of administration, management and policy, is not taken into account herein.

2.1 The Surveillance Strategies

The new eventuality of hybrid warfare, a neologism describing centuries-old practices [12], points towards a unified/joint surveillance, if not a unified/joint response system as well, which will encompass three areas: military and civil healthcare, agriculture-plant protection and fishery and livestock breeding. Once the offensive techniques are similar, pooling together surveillance and response (i. e. defense) resources and methods is the only logical, economical and integrated way which will make better use of given resources. There are of course important points of divergence, but this is true even for the most narrow field of human medicine.

Wider and broader healthcare facilities will allow better interplay, enhanced understanding and communication. The resources saved by having one and not three core health institutions (with respective boards, heads of departments, accounting offices and policymakers) will allow funds directed to a network of surveillance outposts throughout the realm, thus reversing the concentration/centripetal tendency in surveillance units imposed by cost considerations. Thus creation of dispersed, primary care surveillance outposts manned with scientists and/or technicians operating rather inexpensive equipment and linked to operations hubs where specialists –equipped with expensive hardware and plentiful means -will be feasible throughout the realm. These outposts will not only provide syndromic surveillance and collection of samples/cases, but they will be able to perform sample processing and detection/ identification/ diagnosis up to a level. This can be enhanced by robust telematics of standard, commercial amenities and costs, in order to avoid the additional cost, delay and, most important, uncertainties of physical transportation of samples, while at the same time, in cases of crisis, the flow towards specialized centers and reference labs will be checked and kept to manageable levels.

2.2 Equipment and Manning

This kind of surveillance structure is based on a number of assumptions and targets with fractious effects to current healthcare establishments such as diagnostic centers and hospitals/clinics. The allocation of resources and the weight given to massive, austere equipment purchase coupled with human recruitment instead of massive investment on ultra-high-tech and fancy hardware (but for the specialist/reference hubs and nods) will discourage competition for the provision of better healthcare, which drives actual costs up at any given time. Diagnostic hardware will be purchased in a competitive basis, which means an alteration of the level and field of the competition off the services provision and off the providers towards the enablers. This in turn might diminish the corruption widespread in providers' competition for end users (such as prescription "incentives" for physicians) while transferring it upstream, where end costs are less and the level is more manageable. Such approaches ("consolidation") have been implemented in other industry areas with mixed results; the capability and knowledge bases of such areas, though, have been successfully preserved in times of shrinking budgets.

The excessively specific tests, developed more as solutions seeking the right questions rather than on a need-to-have basis, answer nowadays with great accuracy and sensitivity very specific questions. The development of new methods, products and services is mainly attuned to profit and achievement/fame and less to robustness under unfavorable conditions, reduced expenses and integration into wider schemes, which would have forwarded standardization, commonality and redundancy. Although in many cases diagnosis may or even must be performed in this, traditional, manner, surveillance may not. It must be adaptable, low-cost, straightforward in means and ends. This is where an interesting bundle of solutions coming from the military field come to prominence. After having declared the biothreat of utmost importance at the turn of the century by the most official source, the White House [13], the US started a quick reaction program smartly addressing the main lines of the biological threat as described perhaps a decade earlier: the keyword of the American program, in all levels-and not just in surveillance –was "adaptability" and a number of aims and milestones were released in 2006 for congressional reporting [14]. The most interesting issues addressed challenges and solutions throughout the spectrum of the evolving threat. For example, there were tasks focused on enhancing immune response by diverse approaches and tasks focused on working independently from the immune response; the latter are critical as the immune response may be suppressed, compromised, diverted or simply become unpredictable as far as its key performance parameters are concerned (initiation, intensity, duration, specificity, success).

Additionally, new methods of vaccination and fast-track production of vaccines against emerging threats in almost real-time, multi-potent vaccines and therapies targeting common and not specific pathogenic mechanisms constituted a reaction platform based on agnostic and fast evolving contingencies, the former reminding the old, empiric medicine. But the most important lines of research, impacting surveillance and diagnosis, where the obviously multi-factorial analysis of the bio-signature of an infection, thus resolving diagnosis faster than with conventional isolation and study of the pathogen and circumventing possible precautions of the perpetrator to alter basic symptomatology and key detection/recognition entities and loci in order to lead to misidentification and erroneous treatment. Although the results of this multifaceted effort are not released up to now, two platforms allowing a very prompt identification sequence were in advanced stages back in 2005. The CB-64 [14] was a genomic, microarray-based platform to detect, assess and implicate genetically engineered biothreats, performing on-array amplification and sequencing. Tackling the extremely wide field of genetically engineered /manipulated pathogens, it was supplemented with the CB-47 [15], an enhanced immunodiagnosis platform which would cover the detection of exposed (and perhaps less exposed) cellular/viral effector molecules AND cell/virion-free effectors, such as various toxins, prions and perhaps viroids as well [16].

Such approaches require a level of competence but also a level of compliance from the personnel involved. Basic or even advanced biomedical skills are a must but are not enough; the operative word in such cases is “vigilance”, which can be taught, but requires unorthodox approaches and entails sacrifices in work routines, strictness, standard opperating procedures/ SOPs and job-related privileges. The concept of high alert is not easily tolerated as a daily routine in the civilian sector, and some characters are even less prone to such behavior and discipline than others-which brings up the notion of multi-factorial screening of staff by human resources officials. The open disregard to authority and compliance is not the main problem, as it can be dealt with; the silent indifference, the undetectable loss of interest, pride and sense of mission is much more threatening, a known issue to millennia-old sentry-duty personnel. A robust and thorough, though subtle way of frequently testing and (dis)qualifying personnel, along with systemic provisions to renew human resources due to age (which downgrades vigilance-oriented performance) and the above-mentioned concept of fatigue and wariness is of paramount importance. Here the paradigm-shift is obvious: typical human resources concepts require the longest possible service for expensively trained and meticulously picked personnel, but in such cases this is counterproductive as minor slips in performance may lead to catastrophic failures.

3. NGS: a Must, an Option or a Luxury?

The Next Generation Sequencing (NGS) revolution, has been anticipated since the mid-90s, with Hollywood movies showing direct PCRs in abundance of primary biosamples (without extraction) able to produce whole genome sequencing in a matter of some hours. It is about deciphering the whole genome of an organism with medically or industrially usuable performance in terms of promptness and affordably; this became ppossible due to the development of new breeds of reagents, instrumentation and software [7]. It is an astonishing achievement for science and technology, but it cannot be used in direct surveillance and detection due to two restrictions: cost, which, being at the high-hundreds of dollars per sample [17] prohibits massive use, and time. Wet process turn-around times of 4 hours are necessary and of 2 hours are desired for operational relevance in time-sensitive incidents.
Enhanced robustness makes high purity for samples rather redundant, but abundance of specimens must exist within the collected samples. The latter is a mere "tacticality", wholly dependent on the intended use and thus prone to negotiation; the cost and time issues, though, are focal. Having said so, one has to admit that WGS is, or is going to become, indispensable as genomically fabricated (not genetically modified) cellular bio-agents will soon become the norm, once organismic engineering is sufficiently funded, at least overtly. The synthetic genomics already project the use of "deleted" genomic space (undergenomes) which retains minimal, housekeeping gene circuitry in organism/cell platforms [18], [19]. This "recycled" space is used for pasting either whole genomes (unitary overgenomes) or large parts of different genomes (combined overgenomes) or even massive assemblies and conglomerates of genes and /regulatory sequences from scores of different primary sources which are consequently edited (compiled overgenomes). the first approach allows very fast times of introducing new, dangerous and unknown populations which can thwart diagnostic algorithms based on symptomatology and clinical signs.

4. Conclusions

The combination of new and rare pathogens, exacerbated by the massive traveling and migration, perplexes vastly the healthcare surveillance and monitoring by expanding the scope of possible pathogens, practically to infinity. The narrowing of the range, a most valuable tool in diagnosis and surveillance is likely to become unattainable; thus the present, high-specificity and high cost assays, performed by specialized personnel using state-of-the –art equipment will be unattainable and unaffordable. Current military diagnostic approaches, fast and hardened to comply with strict windows of opportunity and different, adverse field conditions as they are, remain basically of little usefulness, as they concentrate in very limited ranges of pathogens, usually up to a score. The exception is platforms which allow parallel processing, thus allowing multiple interrogations per sample [20]; these will become of universal usefulness once they acquire enough growth potential and flexibility, so as to adapt to different requirements. Though, a series of currently developed technologies, which allow fast, on-demand and *ad hoc* development of assays for new and/or unknown pathogens has the potential to revolutionize surveillance by greatly expediting the assay development time, thus shrinking development costs. If a common assay platform, or a number of common assay platforms is selected with suitable provisions for upgrades, the assays will be able to evolve using existing hardware and supplies to a large extend. This way the assays become time-relevant due to in-*promptu* development and affordable, due to reduction of the need for expertise at the phase of individual development and to commonality in infrastructure and expendables required for their routine and massive implementation. If this approach is coupled to an enlarged surveillance scope [21], unified to cover plants, animals and humans, as many pathogens transcend the kingdoms' barrier and even more are to do so as health standards deteriorate globally while the pathogens themselves evolve [22], a new breed of surveillance strategies is attainable. This breed, suitable for both civil, security and military applications [23] may reshape or collapse the diagnostics business sector as we know it, forcing difficult choices to decision-makers.

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