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Review Article

Reviewing the US Select Agents and Toxins List with More Efficient Assessment of the Potential Applicability of Microbes in Bioterrorism: The Fungal Agent

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Abstract

A major consideration in Bio security and Bio defence is whether fungi should be excluded from the US government select agents list and other similar documentation. The mortality of spontaneous fungal infection is indeed low, but it is not an important factor for weaponizing a pathogen. The disruptive effect may be the true aim. Low contagiousness of fungi is also desirable to restrict the infection to the intended targets, while simplifying recruitment and training, and the handling of the device. Spore-generating fungi are easier to weaponize than bacteria, the natural occurring forms of which are not readily suitable for weaponization. As technology progresses, the genetic manipulation is not just to augment a microbe's pathogenicity. Once such procedures are established, the microbe is not the weapon, but the vector for a preferably allogenic toxin or bio regulator. The suitability formula proposed for calculating the Weapon Potential of a microbe includes vital parameters such as inoculum size, contagiousness, stability of the agent, inoculation time but others, with operational significance, such as undetectability, availability of medical countermeasures and circumstantial suitability are not mentioned at all. Such pitfalls should be addressed if the formula is to assist decision-makers to focus their vigilance according to available intelligence.

Keywords: weaponization potential; select agents list; allotoxins; fungi

Abbreviations

WP: Weapon Potential; Fs: the Symptomatic Fraction; I: Inoculum; T: Time elapsing; C: Contagiousness; S: Stability

Introduction

The prerogative of secure handling of microbial flora with destructive potential, either after manipulation and optimization or at its natural occurring form, has been a primary bio security concern. The work of Casadevall & Pirofski [1] touched highly sensitive, important and still up to date issues on biological warfare/bioterrorism, which were further addressed in [2]. The major underlying consideration is whether the exclusion of fungi, and of eukaryotes in general, practically out of the US government select agents list is warranted [3,4]. As remarked [1], only one fungus, *Coccidioides*, was originally included to the Select Agents List, followed briefly by *Cryptococcus*; the 2012 version includes neither [5]. *Coccidioides* was included for being a known problem to US military and endemic in the US. Thus, it featured as a handy organism for *in situ* weaponization, either by US services after in-place collection thus evading international attention, or by malefactors aiming to use it locally and thus bypassing border and point-of-entry controls, an attribute rather underestimated.

Facts, Figures and Intends

The mortality of spontaneous fungal infection is indeed low, but the civilian death toll might be considerable, due to the compromised immune status of considerable population groups [1]. Still, in tactical and strategic context, this is rather irrelevant: contrary to the general perception, mortality is not an important factor for weaponizing a pathogen. The use of bio weapons does not target to massive deaths (destructive effect), but also to functional incapacitation of a perceived enemy (disruptive effect) [6,7]. The latter is occasionally the true aim of the perpetrator. The mild effects of most primary mycoses- and mycotoxicoses- diminish the war fighting capacity of military personnel whose concentration and reflexes are of paramount importance [8]. The panic and economic losses entailed whenever occurring epidemics are followed by bioterrorism notions exemplify disruptive effect on civilian targets [6]. Moreover, due to the low priority assigned to such pathogens in Select Agents Lists, the incident and its nature may remain undetected for a crucial time, turning the perceived shortcoming of fungi to an operational advantage.

Similarly, the prolonged incubation period denies prompt medical countermeasures and practically ensures terminal disease, a prospect most alarming and difficult to detect, measure or counter, thus providing a probable propagator with credible deterrence.

Exactly the same is the nature of the low contagiousness of fungi. As reported with the experience of Japan's *Unit 731* in China, highly contagious pathogens pose a threat for fratricide and contamination of own operators and own civilians. In such cases, non-contagious pathogens would be a better instrument to restrain/contain the infection only to the enemy, especially if coupled with good Standard Operating Procedures [6]. In a bioterrorism context, the use of low contagiousness pathogens simplifies the recruitment and training of

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the operators, reduces the time and effort needed for their preparation and simplifies the handling of the weapon, its manipulation, safekeeping, transportation and deployment. Most importantly, it simplifies mission planning [7]. There are only two plausible scenarios where highly contagious pathogens are preferable. The first is the case of defensive bio warfare, where the defendant infects the offender's territory in order to disrupt/destroy the attacking forces and/or the infrastructure that supports them. In such cases, the defendant can cover with prophylaxis his own population and forces and keep his territory disinfected. The other scenario is the case of very distant, practically intercontinental assaults, where distance safeguards the propagator. In bioterrorism, such applications are possible only through highly motivated or ill-informed operators [7], who are either difficult to find (the former) or -even more- difficult to train for handling the pathogen efficiently (the latter). High infectivity, not mortality/lethality and contagiousness are of prime bio warfare/ bioterrorism interest. Thus, alert, detection and response algorithms should take this into consideration.

The advantages of ease of harvesting and growth and robust dispersion phases are correctly assessed by Casadevall and Pirofski [1]. In most cases of select bacterial pathogens, as *Bacillus anthracis*, the natural form of the microorganism is not readily suitable for weaponization. *Bacillus anthracis* strains must be carefully selected and manipulated to be optimized [9] remark. In the same context, it would be very interesting to project what fungi could do with similar treatment.

Moreover, it is rather restrictive to seek to augment a microbe's pathogenicity by genetic manipulation. Once such procedures are established, the microbe is not the weapon, but the vector [8]. A robust, "optimized" microbe, producing an allogeneic toxin [10] or bio regulator upon germination could wreak havoc and escape medical countermeasures exactly because the toxicosis would be incompatible with the clinical picture of the infection [11]. Eukaryotic expression efficiency coupled with ample genetic space in the form of much "spare" DNA plus a highly effective allogeneic eukaryotic toxin/ bio regulator would be a sought for combination [12].

To Fund or Not to Fund?

The facts and prospects just mentioned pose a serious question on preparedness and proactive measures. The first and foremost issue is related with research. Should it be banned or restricted when referring to a fungal pathogen? The obvious, although not widely appreciated and understood- answer is negative. Fungal entities are in many cases ready-to-use agents requiring no special modification. Very little preparation, of people with mediocre microbiology skills and dispersed and austere facilities may produce usable amounts for small-scale tactical or subtactical endeavors. There is no proactive measure and precaution for the selection and use of environmentally available fungi, by a perpetrator; environmental strains are readily and discretely available and more robust compared to clinical strains, although the latter might be more resistant and/or virulent. Better medical countermeasures and surveillance practices, both in terms of medical intelligence and security intelligence, consist the only available option, although of questionable generalized applicability. The issue is more serious with genetically manipulated fungi. Once more, the expertise and resources have long crossed any border of containment and clandestine work may happen in garages or in corporate safe heavens. It is not only an issue of double-use equipment and supplies. With a labor-intensive process and the most basic of biochemical supplies, manipulation can be accomplished and it is a matter of priorities the way its products are to be tested (perhaps under the cover of a small, dirty war). Possible perpetrators and non-state actors have embraced an expertise-intensive approach to diminish their dependence on infrastructure and hardware, which are liabilities for a clandestine operation. Real-life perpetrators either already have or will NOT endeavor to obtain high-tech facilities and recourses and will make do with the absolute basics. Thus, to admit the true bioterrorist potential of fungi may only increase, not decrease, funding for certain aspects of related research otherwise it is as if one prohibits metallurgy to prevent gunfire incidents. The level of technology is adequate for indefinite manufacture, while the mostly affected area would be the manufacture of protective equipment.

Conclusion

The final issue of this commentary is the suitability formula (Equation 1) proposed for the weaponization of microbes in [3] and its validity for calculating the Weapon Potential (WP):

$$WP=F_S SC/IT \qquad \text{(Equation 1)}$$

where *Fs* = the symptomatic fraction of the exposed population, *I* = inoculum used, *T* = time elapsing between the assault and the onset of incapacitating symptoms, *C* = contagiousness and *S* = stability of the agent. These parameters may be regarded as incomplete, with the key parameter of undetectability absent altogether. As mentioned earlier, applications of bio assault differ significantly. Some microbes are better suited for offensive, others for defensive, others still for strategic and some others for clandestine applications [13], all of which weight differently the time parameter. Lethality, pathogenicity, infectivity, virulence and contagiousness are differently weighted in different mission profiles, contagiousness sometimes being a reason for not using a pathogen. The robustness (*S*, stability) of a microbe is related to the applicable dispersion techniques such as spontaneous, instantaneous, overt, covert, pneumatic or pyrotechnic [2]. The available medical countermeasures define applicability parameters of paramount importance when selecting a pathogen for weaponization. Last, but not least, the manipulation potential, which upgrades the possible effects of a given vector, should be taken into consideration, but conditional to the perpetrators' scientific and technological level [14]. Thus, the proposed formula should initially be expanded, to include other relative parameters, and subsequently it could be differentiated to allow differential weighting of the very same parameters according to the specifics of the intended use. Only then the bio security agencies would be able to plot a reliable risk assessment.

Last, but not least, is the issue of surveying and responding to such mutated threats. The approach is very pernicious and at this point the advantage lies with the offender. If no intelligence-based proactive action can be taken, the only promise might be the very fast full exome analysis, detecting in air samples translatable sequences and virtually guessing their possible pathogenicity. Some problems, of course remain un dealt with: Since environmental flora is used as vector, extremely robust surveillance algorithms should be developed as a first alarm tier, which would result in active collection of samples and testing. Technological breakthroughs, in the cost, duration and multiplicity of the analyses must be accomplished before security and public health institutions may start planning for prompt response to such incidents.

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