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Clinical microbiologists facing an anthrax alert

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Abstract

Microbiological war and terrorist attacks are made to weaken populations by transmitting pathogenic and epidemic microorganisms. These bacteria or viruses are often difficult to diagnose. Anthrax alerts following September 2001 showed that most clinical microbiology laboratories were not adequately prepared, using obsolete diagnostic methods or being too slow to use accurate tools when facing a major threat. Following this period, most microbiology laboratories were prepared for bioterrorism alerts, in order to provide accurate and rapid results, although such events are rare and unexpected. In this review, we describe the organization and preparedness of our clinical microbiology laboratory regarding bioterrorism risk, although its main task is to perform routine diagnostic microbiology tests. To illustrate the difficulties, we briefly describe an anthrax alert.

Keywords: Anthrax, biological warfare, biosafety, bioterrorism, clinical microbiology
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Introduction

Since September 2001, it has been obvious that clinical microbiology laboratories should be prepared for the danger of sudden intentionally caused or natural epidemics. They must be ready to quickly identify agents that are rare and difficult to recognize, because they are not usually encountered, and bioterrorism attack is one of these threats. The pathogens that are considered to be 'bioterrorism agents' are described in the first article of this issue, and, as outlined in this article by Grobusch *et al.* [1], anthrax represents one of the major threats.

For most clinical microbiologists, anthrax was a Gram-positive bacillus affecting mainly animals, and thus mainly concerning veterinary microbiologists. It was also known that this bacterium is capable of forming spores that can persist in the soil for years, and that humans could be infected only rarely, except in a few well-defined endemic countries. Therefore, it was difficult for clinical microbiologists to recognize *Bacillus anthracis*. Indeed, although culture of *B. anthracis* is straightforward, identification at the species level was a major challenge until the availability of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) and corresponding databases, given the significant relatedness between *B. anthracis* and other *Bacillus* species. For laboratories using Bruker MALDI-TOF MS, a dedicated database for 'bioterrorism agents' is available and mandatory. Without this additional database, diagnosis may be delayed, owing to the absence of *B. anthracis* in the routinely used MALDI-TOF MS database, and false identification has been documented recently for one case [2]. Even the identification of a strain with 16S rDNA PCR and sequencing did not allow the different species of *Bacillus* to be distinguished, as *B. anthracis*, *Bacillus cereus* and *Bacillus thuringiensis* are closely related [3]. Now, most clinical laboratories are using the extended biosafety agents Bruker database, which greatly facilitates the identification of the above organisms as well as other bioterrorism agents, such as *Francisella tularensis*. As these agents are uncommon and rarely detected in human samples routinely received in diagnostic laboratories, clinical microbiologists need to be prepared to be able to adequately face any potential alert (Table 1).

TABLE 1. Some pathogens considered to be 'bioterrorism agents'

Bacillus anthracis
Brucella species
Coxiella burnetii
Francisella tularensis
Yersinia pestis
Smallpox virus

After the terrorist attacks of September 2001, we realized that not all diagnostic laboratories were equally prepared for a bioterrorism event, and that the microbial diagnostic methods were often obsolete, slow, and/or inaccurate. Thus, selected laboratories had to implement the required preparedness level and tools to anticipate the threat and to be able to detect the possible agents, such as *B. anthracis*, in different types of sample, including environmental samples, and to detect a putative genetically modified agent.

Before 2001, nothing was ready, as if we had forgotten that, already in the Middle Ages, bodies infected by plague were used for transmitting the disease to enemies (see the historical review in this issue by Barras and Geub [4]). The aim was to weaken populations by transmitting diseases, infecting their cattle, or contaminating their soil and cultures, and so to create a public panic. Thus, major goals of clinical microbiology laboratories when facing a suspected sample are: (i) to be able to detect the agent in the rare event that it might be present; (ii) to reassure the authorities that the examined sample is not harbouring the sought pathogenic agent when it is indeed absent; and (iii) to handle the specimen according to specified rules, to avoid dispersion and transmission to laboratory technicians. Indeed, bioterrorism agents are generally highly lethal and stable in the environment, and are often infectious via aerosols and/or highly infectious (low infectious dose) [3].

For most conventional pathogens detected routinely by clinical microbiologists, the classic approaches of direct examination and culture on axenic media are sufficient. However, for microorganisms that are difficult and dangerous to handle (such as *B. anthracis*, *Yersinia pestis*, *Brucella* species, *F. tularensis*, and *Coxiella burnetii*), molecular diagnosis represents the most accurate, sensitive and specific tool, as well as the safest approach to be used for their detection. Detailed protocols should be available to provide an optimal level of preparedness, because, by definition, these events are unexpected. Moreover, to achieve high reproducibility, specificity, and sensitivity, internal and external quality controls must be performed on a regular basis. Nowadays, it is possible to develop PCRs without using dangerous real 'positive controls' by using plasmids that will serve as positive controls for the PCRs. The danger associated with the preparation and use of positive controls is thus drastically reduced. Moreover, for the suspected sample, one can inactivate the specimen in order to handle harmful samples after extraction of nucleic acids.

In practice, in large diagnostic laboratories such as ours, a significant level of preparedness has been developed to enable recognition of the problem at the very beginning, providing the opportunity to respond to this threat with the highest level of accuracy and without delay.

In this review, we report an anthrax alert as an example, and we use our past experiences to describe the organization and the technological tools that we implemented to face such alerts.

An Anthrax Alert in April 2007

In April 2007 at 10:45 a.m., our laboratory received a telephone call from the fire department regarding an anthrax alert. A previous alert had occurred >3 years earlier, and no specific, regular training had been implemented. However, internal procedures were available through our intranet-verified documentation system, allowing us to quickly fill the information and preparedness gap. These protocols include descriptions of reagents, nucleic extraction procedures, primer and probe sequences, concentrations of reagents used, and positive controls.

According to our internal procedure, before the arrival of the sample, and immediately after having received the initial call, the person on duty informed the biosecurity officer of the hospital and of the laboratory, the head of microbiology, the head of molecular diagnostics, and the heads of all laboratories located close to the biosafety 'bioterrorism' laboratory. Before the sample arrived, protocols were quickly read again by the clinical microbiologist in charge and two senior laboratory technicians requested to handle the specimen. The biosafety laboratory was also checked for the presence of active biocides and all other necessary supplies.

At 12:55 p.m., the sample arrived safely, brought by a firefighter (Fig. 1a). As soon as the sample arrived, the supervisor and one of the technicians allocated to this task obtained additional information on the nature of the sample, and performed a first external disinfection. Once the announcement sheet had been completed with all administrative information, the glove box containing the envelope was transported to the bioterrorism laboratory under the biosafety flow hood. The envelope could not be seen, as several layers of plastic bags and absorbing paper surrounded it. To work in the biosafety level 3 laboratory, the three persons wore dedicated outerwear, consisting of a waterproof suit, waterproof gloves, hair and shoe protectors, and a 3 M mask (Fig. 1b). As these three persons—members of the routine staff—had to be dedicated to this unexpected task, part of the