Lessons to be Learned from Recent Biosafety Incidents in the United States

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ABSTRACT: During recent months, the Centers for Disease Control and Prevention (CDC) announced the occurrence of three major biosafety incidents, raising serious concern about biosafety and biosecurity guideline implementation in the most prestigious agencies in the United States: the CDC, the National Institutes of Health (NIH) and the Federal Drug Administration (FDA). These lapses included: a) the mishandling of *Bacillus anthracis* spores potentially exposing dozens of employees to anthrax; b) the shipment of low pathogenic influenza virus unknowingly cross-contaminated with a highly pathogenic strain; and c) an inventory lapse of hundreds of samples of biological agents, including six vials of variola virus kept in a cold storage room for decades, unnoticed. In this review we present the published data on these events, report the CDC inquiry's main findings, and discuss the key lessons to be learnt to ensure safer scientific practice in biomedical and microbiological service and research laboratories.

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For Editorial see page 316

During June and July 2014 three biosafety mishaps occurred and were reported by the Centers for Disease Control and Prevention (CDC). These incidents raised serious concern about biosafety and biosecurity guideline implementation within the most prestige agencies in the United States: the CDC, the National Institutes of Health (NIH) and the Federal Drug Administration (FDA).

Biosafety incidents emphasize the need for a biosafety management program, which should be implemented concurrently with a culture of safety

The first event was brought to public attention by the CDC's announcement that a breach in handling *Bacillus anthracis* spores involving the unintentional release of potentially viable *Bacillus anthracis* spores on its campus in Atlanta, GA, potentially exposed dozens of employees in three biosafety level 2 (BSL-2) laboratories [1]. In this case, a scientist in the Bioterrorism Rapid Response and Advanced Technology (BRAAT) laboratory prepared protein extracts from a panel of eight bacterial select agents*, including *Bacillus anthracis* spores [2]. The incident occurred during the preliminary assessment as to whether MALDI-TOF mass-spectrometry could provide faster detection. The BRAAT laboratory used, under BSL-3 containment conditions, a method for protein extraction that had been previously optimized for Brucella species in another CDC lab – the Bacterial Special Pathogens Branch (BSPB) laboratory. In this extraction procedure, the organism is treated with ethanol, then with 70% formic acid for 10 minutes, followed by the addition of 100% acetonitrile after which it is incubated at room temperature. The BSPB method incorporates a sterility check of the extracts after 10 minutes of incubation in the formic acid/acetonitrile solution. Those samples are incubated for an additional 48 hours and then examined for growth. This extraction method was never verified by the BRAAT laboratory for its efficacy in inactivating the select bacterial agents used in this case. In the BSL-3 BRAAT laboratory, each protein extract sample had been divided into two aliquots: one filtered through a 0.1 µ apparatus and the other not filtered. All filtered and unfiltered samples were checked for sterility after only 24 hours and the protein extracts were transported to the BRAAT BSL-2 laboratory. It should be noted that due to a mechanical malfunction in an autoclave the sterility test agar plates were kept for a week in the BSL-3 laboratory incubator. During that week, the protein samples from all eight bacterial select agents were processed for MALDI-TOF in the BRAAT BSL-2 laboratory and transported to another two CDC BSL-2 laboratories – the BSPB and the Biotechnology Core Facility Branch (BCFB) – where they were analyzed by mass-spectrometers. On 13 June 2014, the BRAAT BSL-3 staff noticed growth on the sterility test plates that were kept for one week in the incubator (the same plates that they checked after 24 hours and found at that time to be negative). This growth indicates that the samples that were tested for sterility still contain viable spores.

The incident was immediately reported, all MALDI-TOF plates and protein extraction samples were collected and transferred back to the BRAAT BSL-3 laboratory, the two

*Select agents are microorganisms or biological toxins that have been declared and listed by the U.S. Department of Health and Human Services (HHS) or by the U.S. Department of Agriculture (USDA) as having the potential to pose a severe threat to public health and safety

mass-spectrometers were removed from the BSPB and the BCFB BSL-2 laboratories, and more than 70 employees were given prophylactic antibiotic treatment due to the potential exposure to anthrax.

During the CDC investigation of the first event, a second biosafety breach was discovered [2]. The second case involved inadvertent cross-contamination of a low pathogenic avian influenza A (H9N2) virus with a highly pathogenic avian influenza A (H5N1) virus and the subsequent shipment of the contaminated culture to an external high containment laboratory in the U.S. Department of Agriculture Southeast Poultry Research Laboratory (SEPRL) [3]. The contamination of H9N2 with H5N1 occurred in the CDC Influenza Division at the Virology, Surveillance and Diagnostics Branch (VSDB) BSL-3 laboratory. Both H5N1 and H9N2 viruses were cultured concurrently in the same biosafety cabinet and the virus stocks were stored for future use. In response to a request from the SEPRL, an aliquot of the H9N2 virus was sent from the VSDB laboratory to SEPRL on 12 March 2014. Since the H9N2 strain is not a select agent and the VDSB laboratory was unaware that it had been contaminated, select agent transfer procedures were not followed. On 23 May 2014, SEPRL notified the VSDB that it had identified H5N1 virus (a select agent) contamination in the H9N2 sample. The VSDB lab subsequently confirmed the contamination but did not notify the supervisory chain of command and CDC leadership. The incident was reported to the CDC internal select agent program and to CDC management only on July 9 as a result of the "Anthrax case" investigation.

Transportation and shipment of all biological agents are extremely hazard-prone pathways and require special attention

The third event was reported on 1 July 2014 when the NIH notified the appropriate regulatory agency, the Division of Select Agents and Toxins (DSAT) of the CDC, that employees incidentally discovered 12 boxes containing a total of 327 vials of various biological agents such as dengue, influenza, Q fever, rickettsia, 6 vials of variola virus and 10 unknown unlabeled samples [4]. The vials appear to date from 1946–1964 and were kept in a regular unsecured cold storage room in a FDA laboratory located on the NIH Bethesda campus. The FDA took charge of the laboratory from the NIH in 1972, along with the responsibility for regulating biologic products. The vials were discovered during preparations for the lab's move to the FDA's main campus. Upon discovery, the vials were immediately secured in a CDC-registered select agent containment laboratory in Bethesda. Later, on July 7, the 6 variola vials and the additional 10 unknown samples were transported to CDC's high containment facility in Atlanta. Overnight polymerase chain reaction (PRC) testing done by the CDC in the BSL-4 lab confirmed the presence of variola virus DNA, following a viability test that revealed the presence of live variola virus in two of the samples [4,5]. Since variola virus is a restricted agent, CDC notified the World Health Organization (WHO) about the discovery and invited the WHO to participate in the investigation. To top the severe safety mishap, this incident clearly violates international agreements regarding the storage of variola viruses after the eradication of smallpox.

MAIN FINDINGS OF THE CDC INQUIRY

Following the anthrax event the CDC launched an inquiry. They concluded that the main contributing factor was the lack of an approved study plan by the BRAAT laboratory scientific leadership to ensure that the research design was appropriate and met all safety requirements [2]. However, there were other contributing factors, namely: a) the use of unapproved sterilization techniques, b) the transfer of material not confirmed to be inactive, c) the unnecessary use of pathogenic *B. anthracis* when non-pathogenic strains would have been appropriate for this experiment, d) inadequate knowledge of the peer-reviewed literature, and e) lack of a standard operating procedure (SOP) or process for inactivation and transfer to cover all procedures performed with select agents in the BRAAT laboratory.

The BRAAT laboratory used a sterilization technique that had been modified by the BSPB laboratory but was not approved for bacterial spores. The BRAAT laboratory modified the methods of the BSPB laboratory to enable comparison between filtration and non-filtration. It should be noted that this modification was not aimed to assure sterility but to assess the effects on the MALDI-TOF findings. The BRAAT BSL-3 team was not familiar with protein extraction techniques and did not conduct a preliminary literature study to mitigate risks inherent in this procedure. Filtration has been recommended for inactivation of pathogenic bacteria including *B. anthracis* in preparation methods for MALDI-TOF [6,7]. While the chemicals used to process the samples differ in the two publications, both required filtration of *B. anthracis* material with a 0.1 μ filter to remove spores. Drevinek et al. [6] concluded that the formic acid method (as used by the BRAAT laboratory) did not sterilize *B. anthracis*. Moreover, the incubation period was also reduced from 48 hours to 24 hours – without scientific support. Only after the incident was discovered were efficacy tests undertaken to assess the compatibility of formic acid and acetonitrile treatment to inactivate *B. anthracis* spores. Those tests were conducted at the CDC and in an independent laboratory, the Laboratory Response Network (LRN) at the Michigan Department of Community Health (MDCH). Findings from both the CDC internal study and the MDCH indicate that the formic acid and formic acid/acetonitrile treatments were effective in inactivating vegetative cells of *B. anthracis*. On the other hand, the formic acid and formic acid/acetonitrile treatments did not inactivate all *B. anthracis* spores, demonstrating low efficacy of only 3–4 log reduction in spore viability. It should be

noted that sterility tests with a starting suspension of $5x10^4$ *B. anthracis* spores, as cited in the CDC report [2], which results in 4 viable colony forming units, cannot meet the basic criteria of sterility and cannot be used for risk assessment of higher spore concentrations. The inquiry report also uncovered several biosafety breaches regarding all aspects of laboratory practice in the BRAAT laboratories: lack of training, missing standard operating procedures for sterility verification and for sample transportation between different biosafety levels laboratories, unlocked select agent freezers, presence of select agent samples in unauthorized laboratories, and improper use of disinfectants. In response to the inquiry findings the CDC director ordered the BRAAT to cease all laboratory activity on 16 June 2014, to be resumed only when the facility has fully adopted and met all the required biosafety demands.

The CDC conducted an internal review of the second incident [3]. The internal review committee concluded that the cross-contamination occurred at the VDSB BSL-3 laboratory due to the failure of laboratory scientists to adhere to established best practices and the absence of approved laboratory team-specific SOPs for the work being done. Reverse transcription-polymerase chain reaction (RT-PCR) test conducted by the VDSB laboratory confirmed that both the first and the second passages of H9N2 virus were contaminated with H5N1 virus originated in Vietnam that had been cultured on the same day as the H9N2 virus. The H9N2 second passage, which had been sent to SEPRL, revealed a 1000- to 10,000-fold increase in the signal for the H5N1 contaminant when compared to the first VDSB passage sample. Since different strains of influenza are manipulated in the same VDSB BSL-3 laboratory suite, a strict protocol should have been followed to prevent cross-contamination. The VDSB team did not have a proper written SOP but claimed to adhere to established best practice procedures, such as cleaning and disinfection procedures when changing from one strain to another and use of different reagent stocks for each strain. However, the internal review found that on the days that both H5N1 and H9N2 strains were handled the scientific team had entered and left the BSL-3 suite within 51 minutes, an exceptionally short period considering that laboratory scientists are required to shower on their exit from the suite before changing into street clothes. Therefore, the time that these staff members performed the cell culture procedure was substantially shorter than the 1.5 hours that would have been required if the established best practices had been followed. This event prompted harsh criticism since the team leader in the VSDB laboratory failed to report it immediately to a supervisor. In addition, the reviewing committee found that email exchanges between the team leaders in the VSDB and SEPRL did not show any evidence on concern regarding the select agent. Nevertheless, even if a select agent had not been involved, the recognition that transfer of a cross-contaminated virus had occurred would have warranted that the team leader report the incident to the VSDB branch chief immediately. The VSDB branch chief could then have informed the other infectious disease branch laboratories to ensure that the contaminated virus stock was not being used and was under select agent inventory control. In the concluding report the review committee pointed to the need for reviewing or creating several SPOs to document laboratory procedures to be taken for each strain, the absence of viral batch records, and the absence of written shipment logs of all samples to third party laboratories. All viral stocks that were prepared after January 2014 including all the samples that were shipped to other laboratories are to be screened in order to verify that they are not cross-contaminated. In response, the CDC closed the laboratory involved, until improvements of safety and security are implemented.

Furthermore, the CDC director issued a CDC-wide moratorium on all biological material leaving any CDC BSL-3 or BSL-4 laboratory [8,9] until additional adequate and approved safety measures are shown to be in place, including reviewing of policies and procedures for laboratory safety and security in all CDC BSL-3 and BSL-4 laboratories. For that purpose, two biosafety working groups were established: an internal biosafety working group under the direction of the CDC director of Laboratory Safety, and an external advisory group on biosafety comprising leading scientists and biosafety experts which will serve as a working group of the Advisory Committee to the CDC director.

Cross-contamination of samples is a high risk factor in laboratories that use multiple microorganisms or species with different pathogenicity

The root cause of the third safety event was non-strict inventory follow-up that failed to detect unregistered samples for decades [4,5,9]. The samples had most likely been stored in the cold room by NIH personnel years before the laboratory had been transferred to FDA responsibility in 1972. Although several efforts and campaigns had been initiated on the NIH campus to detect lost or old select agent samples, those campaigns focused on freezers and did not include standard cold rooms. It should be mentioned that once the samples had been found, both the NIH and the FDA acted appropriately and the event was reported and handled as required. In response to this event the FDA commissioner issued a call for an inventory check of all FDA cold rooms and freezers.

DISCUSSION

Taken together, these events reflect a disturbing pattern of behavior, which the CDC director termed a "lack of culture of safety" in his testimony at the Energy and Commerce Committee hearing on these events [9]. This stern statement is supported by the fact that similar events had been reported in the last decade but the lessons learnt were not implemented

within the agencies whose biosafety guidelines serve as a gold standard for most scientific institutions in the Western world [10,11].

During the last 10 years there have been hundreds of biosafety-related events [12], some of them sharing the same cause of the recent events [2,9]. In a prior incident in 2006, CDC's BRAAT laboratory transferred vials of anthrax DNA to the Lawrence Livermore National Laboratory (LLNL) as well as to a private laboratory. The BRAAT laboratory assumed that they had inactivated the samples, but upon receipt and testing of the samples at LLNL, viable *B. anthracis* was detected. The BRAAT laboratory implemented new quality assurance procedures to ensure non-viability of DNA preparations of select agents and developed policies that require the signature of the lab's principal investigator prior to shipping or transferring DNA derived from bacterial select agents. These standard procedures were neglected during the current incident, which did not specifically involve preparation of DNA for transfer. Also in 2006, DNA preparations shipped from another CDC laboratory were found to contain live *Clostridium botulinum*, attributed to inadequate inactivation protocols. Similar to the second event, the first case of human cowpox in the USA occurred in a laboratory that had ceased conducting orthopox studies and was focusing on a non-orthopox virus [13]. In that case the CDC inquiry found that freezer handles, pipettes in the biosafety cabinet, and a box with non-orthopox viruses were contaminated with cowpox. A recent CDC report summarizing 14 cases of orthopox-related laboratory-acquired illness (LAI) demonstrated cross-contamination in 3 of the 14 cases (21%). The resultant LAIs were not related to the virus intended for study [15]. In 2009, newly available test methods showed that a strain of Brucella, thought to be an attenuated vaccine strain and previously shipped to LRN laboratories as early as 2001, was not the vaccine strain. The vaccine strain is not considered a select agent, while the strain that was actually shipped is [2,9].

These events emphasize the need for a biosafety management program, which should be implemented concurrently with a culture of safety in order to prevent the reoccurrence of biosafety incidents. Such a program must thoroughly map the labs as a facility and mark their routine. Updated SOPs should be established and reviewed by peers. Focus should be placed on comprehensive basic training for both scientific and supportive personnel, on annual refreshment courses, periodic exercises, and special briefings on incidents (local and international) and review of the findings. In any organization, routine work can drive personnel to indifference, which might lead them to take short cuts or breach protocol, culminating eventually in an accident. The biosafety program should mandate workers to report any accident, near-missed events, and any obstacle in the laboratory that in their perception could cause an accident. All workers' reports should be reviewed as if an incident had occurred, together with implementation of preventive steps.

Special attention should be directed to inactivation protocols. Training courses should focus on the difference between disinfection and sterilization, and when each method is required. The limitations of each method – chemical, physical (i.e., filtration and autoclaving) and radiological – should be emphasized. Each laboratory must define which methods are compatible with the biological agents that are studied in its facilities and adopt strict inactivation protocols. Validation of an inactivation protocol should be performed using the most persistent relevant biological agent, demonstrating that it is free of all living organisms. It is recommended that safety margins be included for each inactivation protocol by limiting the biological agent's maximal concentration below the proven and validated level and by validating proper incubation time for sterility conformation. Firmer SOPs are needed to ensure sterility and proper documentation of the procedure. Transportation and shipment of any biological agent are extremely hazard-prone pathways and necessitate special attention. SOPs of inactive biological material must include a reference for the inactivation procedure that had been used and precise details of the dispatching and receiving laboratories. It is recommended that the transportation of agents or inactive biological specimen be authorized by the responsible investigator at each lab prior to shipment.

Cross-contamination of samples is a high risk factor in laboratories that use the same instruments and biosafety cabinets for multiple microorganisms or species with different pathogenicity. In general it is recommended, if feasible, that each organism be treated in a dedicated zone. When separation is not possible, strict adherence to standard microbiological practice is crucial. High pathogenic strains should be kept in different storage devices that are well marked and registered. The concurrent handling of both species must be executed carefully, beginning with the low grade pathogenic organism, using an adequate disinfection procedure when strains are switched as well as separate reagents in such cases.

Reliability is crucial for every organization's reputation, which is easily compromised. The biosafety program should involve all management levels within the organization. A management notification policy must be followed after an accident occurs or is detected, including notification to external supervising entities, if they exist. This applies to both select and unselect agents. The compatibility and updating of safety procedures and guidelines should be assessed by the scientific staff, the biosafety officials and the management, based on the most updated studies and publications. It is recommended that an internal advisory group dedicated to safety in science also screen all activity on the campus on an annual basis.

In conclusion, a biosafety program should be led by the head of the institute. Establishing and maintaining a culture

of safety involves every scientist, physician and other personnel, demands high awareness and cooperation, and must be driven by the institution management. There is no substitute for adherence to up-to-date protocols, communication policies and transparency.

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