Comparison and Analysis of Biological Agent Category Lists Based On Biosafety and Biodefense

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Abstract
Biological agents pose a serious threat to human health, economic development, social stability and even national security. The classification of biological agents is a basic requirement for both biosafety and biodefense. We compared and analyzed the Biological Agent Laboratory Biosafety Category list and the defining criteria according to the World Health Organization (WHO), the National Institutes of Health (NIH), the European Union (EU) and China. We also compared and analyzed the Biological Agent Biodefense Category list and the defining criteria according to the Centers for Disease Control and Prevention (CDC) of the United States, the EU and Russia. The results show some inconsistencies among or between the two types of category lists and criteria. We suggest that the classification of biological agents based on laboratory biosafety should reduce the number of inconsistencies and contradictions. Developing countries should also produce lists of biological agents to direct their development of biodefense capabilities. To develop a suitable biological agent list should also strengthen international collaboration and cooperation.

Background
Biological agents include bacteria, viruses, fungi and toxins that cause infection, allergy, toxicity or other hazards to human health, and also pose a serious threat to economic development, social stability and even national security. The classification of biological agents is a basic requirement for biosafety and the development of biodefense capabilities. Biological agent classification can be based on two measures: laboratory biosafety and biodefense considerations. For laboratory biosafety assessment, the main consideration is the ability of biological agents to cause disease and the risk of exposure in laboratory accidents. For biodefense assessment, the main consideration is the potential for biological agent weaponization, terrorism and the harm associated with deliberate release [1]. Biological agents category lists also have other purposes, such as the Select Agents and Toxins List of the United States [2] and the Australia Group list of human and animal pathogens and toxins for export control [3]. However, their principle use is for biosecurity to avoid bioterrorist obtaining or abusing biological agents.

The study and handling of biological agents carries with it the threat of biological agents not only includes naturally occurring and emerging infectious diseases [9], but also biological weapons and bioterrorism [10]. In 1999, the US Centers for Disease Control and Prevention (CDC) defined and published three groups of potential bioterrorism biological agents in the category list based on assessment of the threat level of biological agents [11,12]. Following the terrorist attacks of 9.11 and the anthrax mail event in 2001, the European Commission formed a task force on bioterrorism, which became operational in May...
2002. As part of its effort, the Commission was tasked with developing lists of agents for which specific activities should be undertaken to improve preparedness in the EU, as a result two groups of potential bioterrorism biological agents were defined [13,14]. Similarly, Russia evaluated the potential bioterrorism agents and identified three groups of potential bioterrorism agents [15].

In this study, we compared and analyzed the biological agent laboratory biosafety and biodefense category lists and the defining criteria according to specific regions and countries.

**Methods**

The Biological Agent Laboratory Biosafety Category Lists and the category criteria of the WHO, NIH, the EU and China as well as the Biological Agents Biodefense Category List and criteria of the CDC, the European Union and Russia were obtained via internet sites and publications. Biological agents included bacteria (*Rickettsia*, *Chlamydia*), viruses, toxins, fungi and protozoa. Toxins such as ricin, tetrodotoxin, conotoxin, fungi such as *Coccidioides immitis*, and protozoa such as *Cryptosporidium parvum* were included in some category lists, but are not listed in this study.

**Results**

1. **Comparison and analysis of biological agent categorization criteria**

Bacterial agents were divided by the WHO, NIH, EU and China into four laboratory biosafety categories. According to the categorization of the WHO, NIH and the EU, group 1 represents the least risk, while group 4 represents the highest risk. In China, however, categorizations are in reported in the reverse order, with group 4 representing the least risk and group 1 representing the highest risk. In general, the Biological Agent Laboratory Biosafety Category Lists focus mainly on the disease severity, the ability of the agent to spread through the population and whether effective prevention and treatment measures are available. According to the biological agents laboratory biosafety categorization criteria shown in Table 1 (for ease of comparison, the category order of China is reversed), agents in group 1 do not generally cause human disease,

**Table 1. Comparison of biological agent category criterion based on biosafety.**

| Group | WHO[4] | NIH[5] | EU[6] | China[7] |
|-------|--------|--------|-------|----------|
| 1     | A microorganism that is unlikely to cause human or animal disease. | Agents that are not associated with disease in healthy adult humans. | One that is unlikely to cause human disease. | Under normal circumstances, does not cause human or animal disease. |
| 2     | A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposure may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. | Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. | One that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available. | Can cause human or animal disease but under normal circumstances, it does not pose a serious hazard to people, animals or the environment, the risk of transmission is limited, Laboratory infection rarely causes serious illness with effective treatment and prevention. |
| 3     | A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. | Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). | One that can cause severe human disease and present a serious hazard to workers; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available. | Can cause serious human or animal disease. It is relatively easy to spread between people, animals and people, among animals, directly or indirectly. |
| 4     | A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. | Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk). | One that causes severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community; there is usually no effective prophylaxis or treatment available. | Can cause very serious disease in human and animal, including biological agents has not been found in China. |

Note: For ease of comparison, the category order of China is reversed.
doi:10.1371/journal.pone.0101163.t001
have not been identified in China. China includes in this group biological agents that are available. China includes in this group biological agents that have not been identified in China.

Biological agent biodefense categorization is not as common as laboratory biosafety categorization, mainly having been defined by the US CDC, the EU and Russia. Unlike laboratory biosafety criteria, not all agents are divided into four groups. For biodefense categorization, the US CDC and Russia divided biological agents into three groups, with the EU defining two groups. For biodefense categorization, group 1 represents the highest threat, unlike the WHO, NIH and EU laboratory biosafety category lists, for which group 4 is defined as the highest threat.

The US CDC biological agents biodefense evaluation criteria include: (1) can be easily disseminated or transmitted from person to person; (2) result in high mortality rates and have the potential for major public health impacts; (3) might cause public panic and social disruption; (4) require special action for public health preparedness [12]. EU determine the degree of threat associated with biological agents according to the formula $T = (B * M * A * D) + T + C$, where $T$ is the threat level, $B$ is the base score, $M$ is the mortality rate, $A$ is the aerosol spread of ability, $D$ is the ability to spread between people, $T$ is drugs and vaccines for a possible response and $C$ is the production potential. The base score includes the current prevalence in Europe and also refers to the US CDC category list. Ability to spread among people includes the number of susceptible people. Production potential includes the acquisition of potential pathogens, stability, and potential production capacity [14]. Russia divided potential bioterrorism agents into three categories with the main factors to consider including: (1) human sensibility to the microbe; (2) infectious dose

| Biological agents | Biosafety category | Biodefense category |
|-------------------|-------------------|---------------------|
|                   | NIH   | EU     | China | US CDC | EU     | Russian |
| Bacillus anthracis| 2(2)  | 3      | 2     | Category A | Very high threat | Group 1 |
| Yersinia pestis   | 3     | 3      | 3     | Category A | Very high threat | Group 1 |
| Francisella tularensis | 3 | 2      | 2     | Category A | Very high threat | Group 1 |
| Clostridium botulinum | 2     | 2      | 3     | Category A | Very high threat | Group 1 |
| Burkholderia mallei| 3     | 3      | 2     | Category B | Very high threat | Group 1 |
| Burkholderia pseudomallei | 3     | 3      |         | Category B | High threat |         |
| Rickettsia prowazekii | 3     | 3      | 2     | Category B | High threat | Group 1 |
| Rickettsia rickettsii | 3     | 3      | 2     | High threat |         |         |
| Coxiella burnetii | 3     | 3      | 2     | Category B | High threat | Group 1 |
| Brucella species | 3     | 3      | 2     | Category B | High threat | Group 2 |
| Staphylococcus aureus | 2     | 2      | 3     | Category B |         | Group 3 |
| Clostridium perfringens | 2     | 3      |         | Category B |         |         |
| Vibrio cholerae | 2     | 2      | 2     | Category B | High threat | Group 2 |
| Salmonella species | 2     | 3(2)  | 3     | Category B | High threat | Group 3 |
| Shigella species | 2     | 3(2)  | 3     | Category B | High threat | Group 3 |
| Escherichia coli | 2(2)  | 3      | 3     | Category B | High threat |         |
| Chlamydia psittaci | 2     | 3(2)  | 3     | Category B | High threat |         |
| Corynebacterium diphtheriae | 2     | 3      |         | High threat |         | Group 2 |
| Legionella pneumophila | 2     | 3      |         | High threat |         |         |
| Mycobacterium tuberculosis | 3     | 3      | 2     | High threat |         |         |

*NIH laboratory biosafety categorization: Yersinia pestis specifically pgm(-) strains (lacking the 102 kb pigment locus) and lcr(-) strains (lacking the LCR plasmid) are in group 2.

*NIH laboratory biosafety categorization: Francisella tularensis subspecies novicida, strain Utah 112; F. tularensis subspecies holarctica LVS; F tularensis biovar tularensis strain ATCC 6223 are in group 2. EU laboratory biosafety categorization, F. tularensis type A is in group 3, while type B is in group 2.

*Biosecurity categorization: Clostridium botulinum, Staphylococcus aureus and Clostridium perfringens are listed as Clostridium botulinum toxin, Staphylococcal enterotoxin B, epsilon toxin of Clostridium perfringens.

*Chinese laboratory biosafety categorization: Rickettsia spp is in group 2.

*NIH laboratory biosafety categorization: Coxiella burnetii – specifically the Phase II, Nine Mile strain, plaque purified, clone 4 is in group 2.

*Chinese laboratory biosafety categorization: Vibrio cholerae epidemic strains are managed as risk group 2, non-epidemic strains are managed as risk group 3.

*EU laboratory biosafety categorization: Salmonella typhi is in group 3, while other salmonella strains are in group 2.

*EU laboratory biosafety categorization: Shigella dysenteriae type 1 is in group 3, while other strains are in group 2.

*Chinese laboratory biosafety categorization: pathogenic Escherichia coli are listed.

*EU laboratory biosafety categorization: Chlamydia psittaci avian strains are in group 3, while other strains are in group 2. “Chlamydia psittaci” is now reclassified as “Chlamydophila psittaci”.

doi:10.1371/journal.pone.0101163.t002
for infection via aerosol; (3) contagiousness; (4) possible routes of infection; (5) survival in aerosol and in the environment; (6) characteristics of the disease such as severity, lethality and disease period; (7) possibility of mass production of the bioagent; (8) possibility of rapid diagnosis; (9) various means of prophylaxis; (10) various means of treatment [15].

In comparison with the Biological Agent Biosafety Category Lists, the development of Biological Agent Biodefense Category lists requires the consideration of more factors. The development of

### Table 3. Virus biosafety and biodefense category list comparison.

| Biological agents                  | Biosafety category | Biodefense category |
|------------------------------------|-------------------|---------------------|
| NIH                               | EU | China | US CDC | EU | Russian |
| Variola major*                    | 4  | 1     | Category A | Very high threat | Group 1 |
| Ebola virus                       | 4  | 4     | Category A | Very high threat |
| Marburg virus                     | 4  | 4     | Category A | Very high threat | Group 1 |
| Lassa virus                       | 4  | 4     | Category A | Very high threat |
| Machupo virus                     | 4  | 4     | Category A | Very high threat |
| Crimean-Congo hemorrhagic fever virusb | 4  | 4     | Category A | Very high threat |
| Guanarito virus                   | 4  | 4     | Category A | Very high threat |
| Junin virus*                      | 4(2)| 4     | Very high threat |
| Omsk Hemorrhagic Fever Virus      | 4  | 3     | Very high threat |
| Sabia virus                       | 4  | 4     | Very high threat |
| Venezuelan equine encephalitisd   | 3(2)| 3     | Category B | High threat |
| Eastern equine encephalitis       | 2  | 3     | Category B | High threat |
| Western equine encephalitis       | 2  | 3     | Category B | High threat |
| Influenza virus*                  | 3(2)| 2     | 2(3) | High threat | Group 1 |
| Japanese Encephalitis Virus†      | 3(2)| 3     | 2     | High threat | Group 2 |
| Yellow fever virus§               | 3(2)| 3     | 1(3) | High threat | Group 2 |
| Rift Valley fever virus§          | 3(2)| 3     | 2     | High threat |
| Monkey pox                        | 3  | 3     | High threat |
| Kyasanur Forest Virus             | 4  | 3     | High threat |
| St. Louis Encephalitis Virus      | 3  | 3     | High threat |
| West Nile Virus                   | 3  | 3     | High threat |
| Nipah virus                       | 3  | 3     | Category C | High threat |
| SARS- associated coronavirus (SARS-CoV) | 3  | 2 |
| Hantavirus¹                       | 3  | 3(2) | 2     | Category C | High threat |
| Human immunodeficiency virus (HIV) | 3  | 3     | Group 3 |
| Rabies                            | 2  | 3     | Group 3 |
| Dengue virus                      | 2  | 3     | 3     |

*Smallpox is caused by Variola viruses. Variola viruses including Variola major which causes disease with serious clinical symptoms and Variola minor (alastrim) which causes disease with less severe clinical symptoms. NIH laboratory biosafety categorization: Variola major is not listed, but Variola, alastrim and whitepox are restricted to a single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia); EU laboratory biosafety categorization: Variola major and Variola minor are in group 4; Chinese laboratory biosafety categorization: Variola virus and alastrim virus are in group 1.

1 EU biodefense categorization: Congo-Crimean hemorrhagic fever virus is listed.
2 NIH laboratory biosafety categorization: Junin virus candid #1 vaccine strain is in group 2.
3 NIH laboratory biosafety categorization: Venezuelan equine encephalitis is in group 3. Venezuelan equine encephalitis vaccine strains TC-83 and Y3526 are in group 2.
4 NIH laboratory biosafety categorization: Influenza virus is in group 2, 1918H1N1, human H2N2 (1957–1968) and highly pathogenic avian influenza H5N1 strains are in group 3. EU biodefense categorization: lists influenza virus new strains.
5 NIH laboratory biosafety categorization: Japanese encephalitis virus is in group 3. Japanese encephalitis virus vaccine strain SA 14-14-2 is in group 2. EU laboratory biosafety categorization: lists Japanese B encephalitis.
6 NIH laboratory biosafety categorization: yellow fever virus vaccine strain (17D) is in group 3. EU laboratory biosafety categorization: yellow fever virus is in group 1, yellow fever virus vaccine strain (17D) is in group 3.
7 NIH laboratory biosafety categorization: Rift valley fever virus is in group 3, Rift valley fever virus vaccine strain (MP-12) is in group 2.
8 NIH laboratory biosafety categorization: Hantaviruses including Hantaan virus are in group 3. EU laboratory biosafety categorization: Hantaan (Korean hemorrhagic fever) and Seoul virus are in risk group 3, other Hantaviruses are in risk group 2.

PLOS ONE | www.plosone.org 4 June 2014 | Volume 9 | Issue 6 | e101163

for infection via aerosol; (3) contagiousness; (4) possible routes of infection; (5) survival in aerosol and in the environment; (6) characteristics of the disease such as severity, lethality and disease period; (7) possibility of mass production of the bioagent; (8) possibility of rapid diagnosis; (9) various means of prophylaxis; (10) various means of treatment [15].

In comparison with the Biological Agent Biosafety Category Lists, the development of Biological Agent Biodefense Category lists requires the consideration of more factors. The development of Biological Agent Biodefense Category Lists generally takes into account characteristics of individual biological agent, such as their acquisition, production and dissemination capabilities, results of deliberate release and response measures. In addition, the US CDC takes into account the need for special public health response measures, the EU takes into account the epidemiological situation and disease susceptibility factors and Russia takes into account the dose required for aerosol infection.
2. Biological agent category list comparison and analysis

Comparison of the biological agent category lists revealed that the laboratory biosafety risk groups of most biological agents in NIH, EU and China are consistent, although some inconsistencies were identified (Table 2, Table 3). *Bacillus anthracis* was included in NIH biosafety category risk group 2, but was included in risk group 3 according to the EU categorization. Venezuelan equine encephalitis virus and Yellow fever virus were included in risk group 1 of the Chinese biosafety categorization (equivalent to the NIH risk group 4). However, *Shigella* and *Escherichia coli* were included in NIH risk group 3 according to the EU categorization. Eastern and Western equine encephalitis viruses were included in risk group 1 of the Chinese biosafety categorization, but were included in risk group 3 according to NIH and EU categorization, respectively. Monkeypox virus and St. Louis encephalitis virus were included in risk group 1 of the Chinese biosafety categorization, but were included in risk group 3 in the NIH and EU categories. Kyasanur Forest Virus and Omsk Hemorrhagic fever virus were included in risk group 4 according to NIH biosafety categorization, but were included in risk group 3 by the EU.

Most of the biological agents in the biodefense category lists of the US CDC, EU and Russia were found to be consistent. For example, *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Yersinia major*, and Marburg virus were all listed in the highest threat group. However, some inconsistencies were identified. For example, *Bakteriella mallei* was listed in the highest threat group according to the biodefense categorization of the EU and Russia, while the US CDC listed this agent in the second highest category. *Bacteriella pseudazkii*, *Coxella burnetii* were listed in the highest threat group according to the Russian biodefense categorization, but was included in the second highest category by the US CDC and the EU. Influenza virus was listed in the highest threat group according to the Russian biodefense categorization, but was included in the second highest category by the EU, and was not specifically listed by the US CDC.

In general, the grade order of most biological agents of laboratory biosafety category list and biodefense category lists was found to be consistent. Commonly, agents included in a high laboratory biosafety category [such as *Yersinia major*, Ebola virus and Marburg virus] were categorized accordingly at the higher biological defense level and those categorized at lower laboratory biosafety levels [such as *Salmonella* and *Shigella*] were also included categorized at lower biodefense levels. However, some inconsistencies were identified, such as *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis* which were categorized according to the highest biodefense level, but were not included in the corresponding laboratory biosafety level. Similarly, immunodeficiency virus and *Mycobacterium tuberculosis* were categorized according to the highest laboratory biosafety level, but were not included in the corresponding biodefense level. This inconformity is relative to the different purposes and criteria of the two types of category list.

Conclusions

Classification of biological agents based on laboratory biosafety facilitates enhanced biological agent management and reduces the incidence of laboratory personnel infections and environmental contamination. The classification of biological agents based on biodefense provides a focus for improving biodefense capabilities, such as biodefense science and technology layouts and assisting in the prioritization of vaccine and therapy development. In defining the biological agent category list and strengthening biosafety and biodefense, the following aspects require consideration.

1. Biological agent classification based on laboratory biosafety should reduce inconsistencies and contradictions

The WHO has published biological agent laboratory biosafety risk group classification criteria, but has not published the category list based on this classification. Some countries refer mainly to the biological agent biosafety category lists published by the US CDC, NIH or EU.

The category lists and criteria published by the US CDC, NIH, EU or China have certain inconsistencies. For example, in the WHO laboratory biosafety standard, the third risk group is defined as, “not ordinarily spread from one infected individual to another”, but the EU criteria states that, “it may present a risk of spreading to the community”. According to the WHO standard, the NIH and EU list should not include *Mycobacterium tuberculosis*, SARS-CoV and other group 3 biological agents.

In addition, the names of biological agents are occasionally inconsistent. The EU and NIH laboratory biosafety categorization lists the Equine Morbilli Virus, while the same virus is designated as Hendra virus in the Chinese laboratory biosafety category list. These inconsistencies could impede international communication and delay situation management.

2. Developing countries should also have biological agent biodefense lists to direct the development of biodefense capabilities

Bioterrorism and biowarfare poses an enormous threat to humankind. Many biological agents have no effective preventive or treatment measures, and even usable vaccines and drugs often have serious side effects. Therefore, the development of more effective and safer prophylactic and therapeutic measures is urgently required. The United States has launched “Project Bioshield” and other project to strengthen their biological defense capability [16], which is mainly based on the CDC biological agent list. The EU and countries in other regions also attach great importance to the development of biological defense capabilities, based on their respective biological agent lists.

However, bioterrorism and biowarfare threats are not unique to developed countries. Some developing countries are densely populated, with scant biodefense budgets, and bioterrorism would be even more effective in low resourced regions. Developing countries should also have biodefense biological agent lists. Some countries can refer to the biological agent list of US CDC, but this is not a universal list as it may be suitable for the United States. During the developments of Biological Agent Biodefense Lists, developing countries should evaluate characteristics of each biological agent and threats faced, existing biodefense capabilities based on its specific regional conditions. The CDCs of developing country should play an important role in this process and cooperate with other related departments and organizations.

3. The development and revision of biological agent lists requires international cooperation and collaboration

From the differences in the biological agent category list based on laboratory safety or biodefense, it can be seen that worldwide cooperation and collaboration is lacking, the criteria for inclusion on laboratory biosafety lists are not uniform, the names of some biological agents differ between countries, and the list orders are contradictory. In the making and revising of biological agent lists, international level discussion is highly important.

With regards to biodefense, international cooperation is even more absent. Biodefense is often considered as a sensitive field, and it may be simpler to collaborate well in other scientific areas such
as cancer or HIV research. Lessons from such fields could be applied to the area of biodefense collaboration.

The development of laboratory biosafety and biodefense capabilities require shared experience to face the pressing questions of modern-day threat. Cooperation can benefit each other, including further discussions into the making or revision of biological agent category lists.

Author Contributions
Conceived and designed the experiments: DT. Performed the experiments: DT. Analyzed the data: TZ. Contributed reagents/materials/analysis tools: TZ. Wrote the paper: DT. Manuscript submit and revise: DT.

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