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Biosafety Challenges for the Microbiology Laboratory

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Abstract

Microbiology research must be conducted in a fashion that assures the health and well being of the researcher and the safety of the community. This lecture raises awareness of biosafety issues and discusses how the interaction of the pathogen being studied, the person conducting the research, and the practices being used can be manipulated to assure safety. The characterization of pathogens into Risk Groups, how these relate to Biosafety Levels, and the personal practices and laboratory design criteria associated with each Biosafety Level are explained. The importance of preventing or containing aerosols, limiting opportunities for cross-contamination, and taking a flexible multi-component approach to biosafety are emphasized.

Introduction

In their quest to improve human well-being, microbiologists need to be vigilant about their own well being through constant review and emphasis on biological safety in their laboratories. Two recent cases of laboratory acquired diseases in the U.S. have generated renewed concern about biosafety. In the first outbreak, researchers at Boston University Medical Center contracted tularemia (Holzman, 2005). Tularemia causes muscle pain, fever, chills and shotness of breath and has a fatality rate of ~30% if left untreated. The diagnosis was delayed, and the afflicted researchers initially discounted a laboratory related origin because the strain they were studying was (supposed to be) a non-virulent vaccine strain. However, subsequent investigation revealed that their organism was fully virulent. Although all three microbiologists fully recovered, the incident increased public opposition to the building of a new high security BSL-4 laboratory on campus. The second, more alarming incident occurred at a government laboratory near Washington, D.C. In this case, procedures that generated aerosols were conducted on an open bench and infected a researcher on an unrelated project with *E. coli O157:H7 (Bavoil, 2005).* She developed hemolytic uremic syndrome, organ failure, and lapsed in to a coma but has recovered. Since laboratory acquired infections are frequently unrecognized and often unreported, these incidents underscore the need for vigilance on the part of all microbiologists.

The pattern of the international biohazard symbol with its three intersecting rings, is abstract and based on graphic design considerations (Cook, 2001). However, if the three rings are seen as representing "pathogen, person, and practices" (Figure 1), their intersection represents the area where hazards exist. The interaction of the pathogen, the person, and laboratory practices (which include physical barriers and facilities engineering) can be minimized in a variety of ways to assure the safety of microbiological procedures. This metaphor provides the structure for this paper.

The Pathogen

Different species of bacteria present different levels of threat and can be characterized into different risk groups (Table 1) (Richmond and McKinney, 1999). Organisms that are not *normally* pathogenic to humans are placed into Risk Group 1 (RG-1) and can be studied in open laboratories. The vast majority of microbiological research uses bacteria categorized in RG-1. Since almost any bacteria can become opportunistic pathogens (e.g. "food grade" lactic acid bacteria which are safe for human consumption have been implicated in bacterial endocarditis), RG-1 organisms should still be handled using Good Microbiology Techniques (GMTs, as outlined below).

Most foodborne pathogens, ranging from *Listeria monocytogenes* (which only affects healthy people when ingested in high numbers) to *Clostridium botulinum* (which produces the most potent toxin known) are placed in Risk Group 2 (RG-2) because interventions such as immunizations, anti-toxins, or antibiotics are available. Although these organisms *may* have serious consequences, they *rarely* do. While they are seen as posing a moderate risk to the investigator, they pose a low risk to the community. The volume or concentration of the bacterial agent may impact its Risk Group designation. For example, the use of large

Table 1. Classification of microbial agents by risk group.

Risk Group	Defining Characteristic	Relative Risk		Examples[1]
		Investigator	Community	
1	not pathogenic to healthy adults	low	low	B. subtilus, E. coli K-12
2	pathogens, rarely serious, interventions *often* available	moderate	low	most foodborne pathogens, C. *botulinum*, L. monocytogenes *E. coli* O157:H7 *B. anthracis*[2]
3	pathogens, serious or lethal, interventions *may be* available	high	low	*M. tuberculosis*, *Yersinia pestis*
4	pathogens, serious or lethal, interventions *not usually* vailable	high	high	Viruses: Lassa, Ebola, Marburg (no bacteria)

[1] These examples represent subjective assessments which may differ among countries.
[2] Due to security and political considerations, is often treated as Risk Group 3.

quantities or high concentrations of *C. botulinum* increases the risk to the investigator, elevating it to Risk Group 3. Conversely, the use of nonvirulent surrogates, such *C. sporogenes* for *C. botulinum*, *L. innocua* for *L. monocytogenes,* or genetically altered nonpathogenic mutants, can reduce or remove the risk associated with the research. Serious or lethal pathogens, such as *Mycobacterium tuberculosis,* for which interventions may be available but pose high investigator risk are placed in RG-3. The route of probable exposure also impacts Risk Group classification. Organisms which are normally contracted by inhalation pose more risk than those contracted by ingestion or cutaneous exposure. Untreatable lethal viruses such as Ebola pose high risk to the investigator and to the community, are classified as RG-4, and are so specialized as to be beyond the scope of this paper.

The Person

The immunological health status, education, and experience of the microbiologist also impact the hazard associated with the research. For example, the incidence of listeriosis in the U.S. is 0.7/100,000 for the general population, 2.1/100,000 for people over 70 years old, 11.9/100,000 for pregnant women and 70/100,000 for people who are HIV positive (Southwick and Purich, 1996). Assuming equal exposure to the bacteria, pregnancy increases risk by almost 20-fold. Although sex-discrimination laws in the U.S. would prevent pregnant women from being forbidden to work with *L. monocytogenes*, most laboratories have such policies in place. Similarly, where vaccines exist, institutions may make them mandatory for all researchers, or give researchers a choice of vaccination or increased levels of personal protective practices. For example, immunized researchers might work at Biosafety Level-2 (see below), while un-immunized researchers would be required to use BSL-3 work practices. Training and experience are also important factors. The author feels it is as inappropriate for someone without training and experience in general microbiology to work with pathogens.

The Practices

The practices which assure the biological safety of microbiological research can be seen as a pyramid composed of work practices, personal protective gear, and engineering practices. These build on each other; the presence of higher level safeguards should not encourage laxity at lower levels. The CDC estimates that only 20% of acquired laboratory infections can be traced to overt accidents. This means that investigators must always be thinking about the potential of infection by aerosols and indirect contact (i.e., cross-contamination by pens, gloves, notebooks, etc).

"Good Microbiology Techniques (GMTs)" are prerequisite for all other safety measures. GMTs include prohibitions against eating, drinking, smoking, applying cosmetics and mouth pipetting. Microbiologists should wear gloves, wash hands, minimize aerosols, disinfect work surfaces and decontaminate experimental material prior to disposal. Many common laboratory procedures can create aerosols. These include withdrawing samples through septa, pipetting with "blow out" pipettes, vortex mixing, blending,

stomaching, centrifugation, flaming loops, and sonication. Each of these procedure can be done safely if appropriate safe guards are in place. For example, a cotton ball soaked in disinfectant can be placed over a septum, centrifuge tubes that seal with "o" rings prevent aerosols from spinning out, or apparatus can be placed inside secondary containers as simple as plastic bags. Cross contamination by gloves worn outside the laboratory, "laboratory" pens being used for desk work, and spills of pathogens onto "common use" equipment can be serious issues.

Lab coats, gloves, and safety glasses are the minimal personal protective gear for all microbiologists. Cloth lab coats that button up the front may allow contamination of "street clothes;" disposable laboratory coats that close in the back are preferable. Depending on the nature of the organism and the physical barriers in place, it may be appropriate to wear full face shields, surgical masks, personal breathing apparatus, shoe covers, etc.

Engineering practices incorporated into the design of microbiology laboratories can also be critical to the safe conduct of research. These increase as the level of risk from the organism increases. All lab benches should be made of impervious material that is easily disinfected. Facilities to decontaminate experimental material prior to exposure are also required. For research with BSL-2 organisms, locked doors should prevent access by unauthorized persons during experiments and a biological safety cabinet should be available for procedures that generate aerosols. Research on BSL-3 organisms with an inhalation mechanism of infection require that all manipulations be done in a biosafety cabinet in a room that has negative airflow. The exhaust from this laboratory may be filtered to provide additional community protection.

The Interaction of Pathogen, Person, and Practice

The relationship among the risk group of the organism, the biosafety level of the laboratory, work practices and safety equipment is shown in table 2. Although presented as "boxes," there is actually a

Table 2. Relationship of Risk Groups to Biosafety Levels, Practices and Equipment[1] (RESH, 1998)

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
RG-1	BSL-1	Basic Teaching	GMT[2]	Open bench, gloves, lab coats
RG-2	BSL-2	Clinical Research Diagnostic	GMT, Protective clothing, restricted access, Biohazard Symbol	As above and Biological Safety Cabinet for aerosols
RG-3	BSL-3	Special Research or Diagnostic	As above, plus: PPE3, controlled access, negative airflow	As above, and all manipulations under Biological Safety Cabinet
RG-4	BSL-4	Maximum Containment Facility	As above, plus: Airlock Entry Shower Exit Special waste treatment	Class III Biological Safety Cabinet, Pressure suits, Double-ended autoclave

[1] This table illustrated general relationships and should not be used in place of other legal requirements.
[2] Good microbiology techniques.
[3] Personal protective equipment such as respirators.

continuum of actions that can be taken at the level of the person, the pathogen, or the practice that can be manipulated so as to provide safety. There are usually a number of different approaches that can achieve the same level of safety. This paper closes with three examples from laboratories at Rutgers University. 1. When vaccines to organisms contracted by inhalation became unavailable, safety was maintained by implementing the use of a personal breathing apparatus. 2. Aerosols were unavoidable in a study of produce contamination by sprayed irrigation water containing *E. coli* O157:H7. So this study was conducted safely on a laboratory scale inside a Plexiglas chamber. 3. *Mycobacterium tuberculosis* is highly virulent by an inhalation route, so when a less experienced student wanted to study potential anti-tubercular agents (Montville, et al. 1999), nonpathogenic *M. smegmatis* was used as a surrogate to achieve the desired degree of safety.

Conclusion

Safety is a pre-requisite to good microbiology research. The most critical component is awareness of the issue. Once aware, microbiologists can conduct virtually any experiment safely if proper consideration is given to the nature of the pathogen being studied, the person who is conducting the experiments, and the laboratory and engineering practices being used.

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