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Containment evidence Based- Biosafety :- Effectiveness of Microbiological Measures for the handling of Mycobacterium isolates in the laboratories

Zaki E. R[*], Riad E.M[**], Dalia M. Mohsen[*] and Amany N.Dpagh[**]

[*]Buffaloes diseases Department and [**]Bacteriology Department - Animal Health Research Institute

Abstract

Tuberculosis is a severe infectious disease caused by species of the *Mycobacterium tuberculosis complex. M. bovis* is responsible for pulmonary disease in bovin and sometimes to tuberculous mastitis with passage of tubercle bacilli in milk.*M. bovis* is responsible for extra-pulmonary infections in human and also pulmonary infections by inhalation of infected droplets through direct contact with infected animals.

Staff working in microbiological diagnostic and research laboratories is likely to be exposed to Infection risk with pathogens. M. species are essentially an airborne Pathogen included in Risk Group 3 according to the international classification and It is transmitted via aerosols or less frequently by accidental inoculation. The definite diagnosis of tuberculosis relies on the isolation and identification of the Mycobacterium in clinical specimens. . It was showed that 80% of all accidents were due to human error and 20% to equipments problems. even if equipments troubles were partially solved by the adoption of appropriate safety equipments in many diagnostic and research laboratories, behavioral factors may be a source of concern. There are numerous records of laboratory-acquired tuberculosis infection through aerosols or skin puncture .

Biological Risk Assessment of Laboratory Activities through Species of the Mycobacterium must be considered.

The bacterial load of infected material (such as sputum specimens and cultures), and the viability of TB bacilli. Route of transmission of TB; the location of the laboratory; The epidemiology of the disease and the patient population served by the laboratory; the level of experience and the competence of the laboratory's technicians in addition to the health of the laboratory's workers. **Biosafety Recommendations for the Contained Use for isolation of Mycobacterium species** a**nd the use of disinfection, inactivation of *M. tuberculosis* isolates and waste management** as well as The Personnel should receive regular updates and appropriate additional training, under the supervision of the head of the laboratory are discussed.

KEYWORDS : :tuberculosis -lab diagnosis - biosafety

Introduction

Tuberculosis is a severe infectious disease caused by species of the *Mycobacterium tuberculosis complex.* This complex includes *M. tuberculosis, M. africanum, M. bovis, M. pinnipedii* and *M. microti* **(Wayne, 1984; Cousins et al., 2003).**

The four first species are human pathogens, *M. bovis* is responsible for pulmonary disease in bovine and sometimes to tuberculous mastitis with passage of tubercle bacilli in milk.

Both *M. bovis* and *M. pinnipedeii* are responsible for human diseases.

M. bovis is responsible for extra-pulmonary infections in human following ingestion of contaminated milk or milk products, but also pulmonary infections by inhalation of infected droplets through direct contact with infected animals. *M. africanum* is responsible for about 20 to 80 % of human tuberculosis in sub-Saharan Africa, but also there are some tuberculosis cases diagnosed outside this continent **(Cousins et al., 2003)**

Staff working in microbiological diagnostic and research laboratories is likely to be exposed to Infection risk with pathogens. Among human infectious diseases, M. species are essentially an airborne pathogen included in Risk Group 3 according to the international classification (Bloom, 1994).

It is transmitted via aerosols or less frequently by accidental inoculation. The definite diagnosis of tuberculosis relies on the isolation and identification of the Mycobacterium in clinical specimens.

Tuberculosis remains the second leading cause of death worldwide, killing nearly 2 million people each year and The global tuberculosis case load appears to be growing slowly.

Behavioral factors are important in the contribution of laboratory-acquired infections. It was showed that 80% of all accidents were due to human error and 20% to equipments problems. even if equipments troubles were partially solved by the adoption of appropriate safety equipments in many diagnostic and research laboratories, behavioral factors may be a source of concern. There are numerous records of laboratory-acquired tuberculosis infection through aerosols and /or skin puncture (Vaquero et al., 2003).

A survey of 56 state and territorial public health laboratories in the USA has examined the status of existing tuberculin skin testing (TST) and assessed the probable laboratory-acquired tuberculosis.

Among 49 laboratories, 13 reported that 21 employees converted TST (period of 4 years). Seven of these 21 lab-workers were reported to have Lab aquired infection (Harding & Brandt Byers, 2000). .

Biological Risk Assessment of Laboratory Activities through Species of the Mycobacterium:-

The challenge for managers of TB programmes and staff at laboratories, particularly in resource-limited settings, has been to interpret the generic risk-group assignments and biosafety levels into specific precautions relevant to a country's activities. As a result, the use of biosafety levels 1–4 when describing the needs of TB laboratories has led to confusion about what precautions are necessary. Decisions about which are the most appropriate biosafety measures for a specific laboratory should be undertaken using an approach based on risk assessment that considers the different types of procedures performed by the laboratory (Miller et al., 1987; Müller,1988). .

Risk assessments require careful judgment: Basically , underestimating risks may lead to biosafety hazards but, on the other hand, safeguards that are more rigorous than actually needed may impose unnecessary burdens – both financial and in terms of human resources – on a laboratory's staff and management.

The risk-assessment approach for the detection of mycobacterium species in laboratory :

- The bacterial load of infected material (such as sputum specimens and cultures), and the viability of TB bacilli.
- Route of transmission of TB; the location of the laboratory;
- The epidemiology of the disease and the patient population served by the laboratory; the level of experience and the competence of the laboratory's technicians in addition to the health of the laboratory's workers.

Determining risks:-

Risk is the combination of the likelihood that a specific hazard will be encountered and the consequences of an event related to that specific hazard. Risks should be identified and categorized, and a determination should be made about which risks need to be controlled or minimized. The analysis of aerosolization risks led to the development of minimum biosafety requirements necessary for performing different procedures in TB laboratories.

Risk precautions levels associated laboratory activities and risk assessment for Mycobacterium:-

Risk level of TB	
laboratory Assessment of risk	Laboratory activities
Low risk	Direct sputum-smear microscopy; preparation of specimens
Moderate risk	Processing and concentration
	of specimens for inoculation on primary culture media;
High risk	(TB-containment laboratory)
	Culture manipulation for identification, High risk of generating infectious
	aerosols from specimens; high concentration of infectious particles

Biosafety Recommendations for the Contained Use for isolation of Mycobacterium species:-

The WHO and the CDC classify Mycobacterium among the pathogens that require a biosafety level 3 (CDC, 1999; WHO and Laboratory Safety Manual, 2004)., For the manipulation of this human pathogen, greater emphasis is placed on the use of primary and secondary barriers to protect laboratory employees in direct contact with the micro-organisms, the community and environment from exposure to potentially spreading of infectious particles.

Based on the risk assessment and according to technical characteristics, safety equipment and work practices, the following recommendations for the contained use of M. tuberculosis are proposed**:**

1. Laboratory work with clinical specimens susceptible to contain species of the Mycobacterium:-

First of all ,The outside of containers used for collecting clinical specimens could be contaminated with tubercle bacilli, therefore the containers and packaging containing clinical specimens, primary or the secondary culture samples or any other material known to contain *M. tuberculosis* should be opened in a class I or II biosafety cabinet (BSC).

- Personnel wearing gloves should disinfect the outside of the container.

- For the laboratory involved in the diagnosis of tuberculosis, direct smear examination and primary culture of specimens require to work in BSL-2 facilities (Belgian Biosafety Server, 2006) .

2. Laboratory working with *M. tuberculosis* cultures:-

Biosafety measures to apply to aerosol producing activities (Schmid et al., 2003).

Activity	Biosafety measures
Falling droplets Acid-fast staining (AFB smear) Opening of primary and secondary culture Opening of wet caps	- All these manipulations should be performed in a class I or class II BSC
Work with inoculation loops Handling of infected animals and animal litter	• performed in a class I or class II BSC • use of disposable plastic loops is preferable • use aerosols-free buckets "safety cups" during centrifugation • opening rotors, buckets or tubes under class I or class II BSC after centrifugation • use of "droplet containment module" • animals should be maintained in isolators • cages should be opened in a class I or class II BSC

3. Infected Animals with *M. tuberculosis* complex species:-

- Non-human primates infected with strains of the *Mycobacterium tuberculosis* complex should be handled using standard precautions in BSL-3 animal facilities, equipment and work practices .

- Harvested samples from infected animals should be handled using standard precautions in BSL-2 facilities .

- Infected rodents (mice, rats, rabbits) can be housed in a BSL-2 containment since they are maintained in isolators.

- However, BSL-3 work practices should be adopted. Manipulations involving opening of cages should be realized in class I or class II BSC.

4. Disinfection, inactivation of *M. tuberculosis* isolates and waste management

The high lipid content of the cell wall confers to the mycobacteria a great resistance to classical disinfectants **(Kunz & Gundermann, 1982).**

The bacilli are generally more resistant to chemical disinfection than other vegetative bacteria. Their resistance to disinfectants is considered intermediate between other non-sporulating bacteria and spores while the acquired multidrug resistance does not seem to modify the resistance to disinfectants .

Efficient disinfectants

1- Quaternary ammoniums inhibit tubercle bacilli .

2- Mercurial compounds are considered to be ineffective against the mycobacteria.

3- phenol. 5%

4- formaldehyde at least ten minutes. 5%

5- glutaraldehyde 2% for 30 minutes exposure .

6- Ethyl and isopropyl alcohols in high concentrations are generally excellent mycobactericidal agents.

7- ethyl alcohol 70% can be used as surface disinfectant.

8- Formaldehyde vapours can be used to disinfect BSC's and facilities.

9- Iodine and ionophores are considered to be effective against mycobacteria and are generally used in combination

General recommendations :

1- Work surfaces should be decontaminated at least once a day with an appropriate disinfectant and immediately after any accidental contamination with infectious materials.

2- Laboratory workers should disinfect their hands after manipulations with an appropriate disinfectant, after removing gloves, and before leaving the laboratory (Schwebach et al., 2001).

Worn gloves and protecting clothes should be autoclaved before leaving the laboratory.

3- Attention should be focused given to waste inactivation,

decontamination by autoclaving or incineration is essential.

An autoclave for the sterilization of contaminated materials should be available in or adjacent to the laboratory.

If the inactivation takes place outside the laboratory (autoclave or incinerator), wastes should be placed in a leak proof bag or an unbreakable and leak proof container (for liquid wastes), sealed and disinfected on the outside before removal from the laboratory.

In addition to the international Biohazard symbol, bags or containers should be adequately labeled to prevent opening before decontamination and the removal of bags and containers should be performed .

4- Mistakes and accidents, which result in overt exposure to infectious materials, should be immediately reported to the head of the laboratory and eventually to the local biosafety officer.

5- Written records of such events should be kept.

6- Personnel concerned by the Mycobacteria activity should be experienced and should receive regular updates and appropriate additional training under the supervision of the head of the laboratory.

Finally, The increase of incidence of tuberculosis in industrialized countries and concomitant emergence of antibiotic multidrug resistance have highlighted the necessity to elaborate specific biosafety measures for manipulation of mycobacterium belonging to the *M. tuberculosis* complex, in diagnostic and research laboratories.

These recommendations are based on a thorough risk assessment taking into account the type of activity.

The adoption of a BSL-2 containment with BSL-3 work practices are recommended for medical laboratories limiting their analysis to Mycobacterium isolation from clinical specimens (i.e. primary culture, microscope examination of smears from clinical specimen, nucleic acids amplification, histological examination).

The work on biological material susceptible to generate infectious aerosols must be performed in a class I or class II BSC and placed in a specific area to be separated from the other bacteriological activities.

The use of a centrifuge equipped with "safety cups" is highly recommended BSL-3 containment, safety equipment and work practices are necessary for laboratories

manipulating positive cultures of the *M. tuberculosis* complex at ends of diagnosis or research work (e.g. biochemical tests, susceptibility testing, subcultures for research work) until validated inactivation of mycobacteria.

The respect of these biosafety recommendations associated with appropriate measures of prevention and/or medical follow-up for laboratory staff should contribute to minimize risks of being infected by Mycobacterium at work and protect environment.

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