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Biohazards of *Mycobacterium tuberculosis*

India

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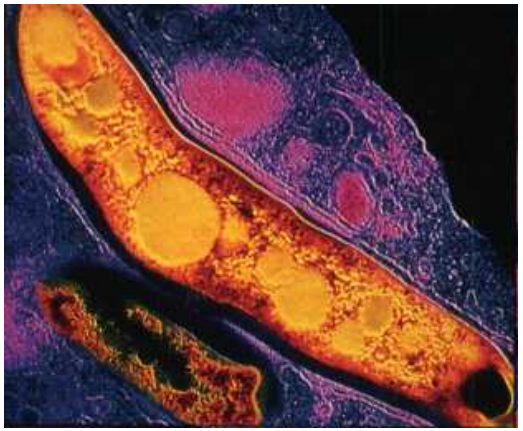
Outline

- History of Laboratory Acquired Infections
- Health Hazards
  - Classical Strains
- Viability
- MDR/XDR *M. tuberculosis*
- Laboratory Hazards
- Recommended precautions/practices
  - Containment
  - PPE
  - Decontamination
  - Inactivation
  - Incident response
- Medical surveillance



History of Laboratory Acquired Infections

- Incidence of TB among laboratory workers working with TB 3 to 5 times greater then laboratory workers not working with TB*
- In a study of 16 laboratorians with traceable exposures:
  - 10 involved poor directional airflow
    - 8 within a lab
    - 2 within a clinic
  - 5 associated with failure in the biosafety cabinet (BSC)
  - 1 associated with an autoclave failure
- In 1993, a nurse acquired TB via a needle stick injury from an HIV/TB infected patient. The nurse did not acquire HIV **

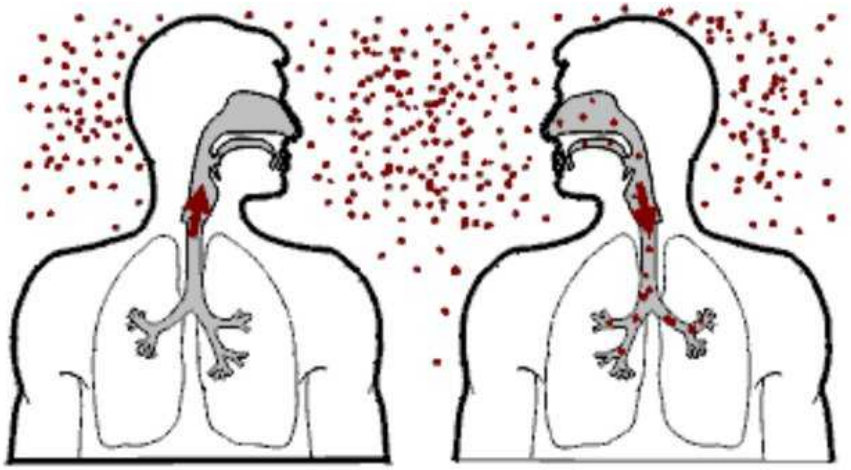
*CDC Report, June 1997
** Kramer et al, 1993

Health Hazards

- *M. tuberculosis* falls into risk group 3
  - H37Ra & Bacillus Calmette-Guerin (BCG) fall into risk group 2
  - H37Rv is a risk group 3 strain
- Infectious dose is very low:
  - ID_{50} 1-10 bacilli
- Routes of infection
  - Inhalation of infectious aerosols
  - Accidental parenteral inoculation
  - Direct contact with mucous membranes
  - Ingestion

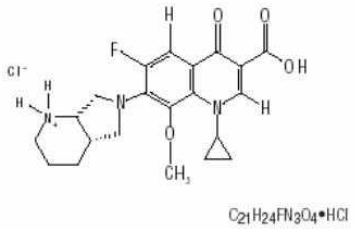
Viability

- M. tuberculosis is fairly stable in the environment
  - 90 to 120 days on dust
  - 45 days on manure
  - 105 days on paper
  - 6 to 8 months in sputum (within a cool dark location)
  - 45 days on cloth material



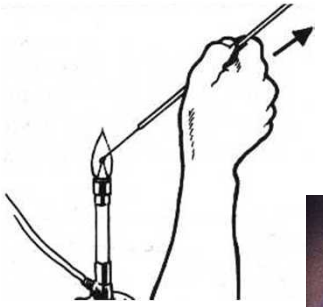
MDR/XDR - TB

- **Multi-Drug Resistant (MDR-TB) is a TB strain showing resistance to at least rifampicin and isoniazid**
- **Extensively Drug Resistant (XDR-TB) is also resistant to rifapicin and isoniazid in addition to fluoroquinolone and at least 1 of the following injectable drugs: capreomycin, kanamycin and amikacin**
- **Infectious dose and routes of infection are believed identical to standard *M. tuberculosis***
- **Viability of XDR and MDR is also believed to be the same but some inactivation tests demonstrated XDR may be less stable**



Laboratory Hazards

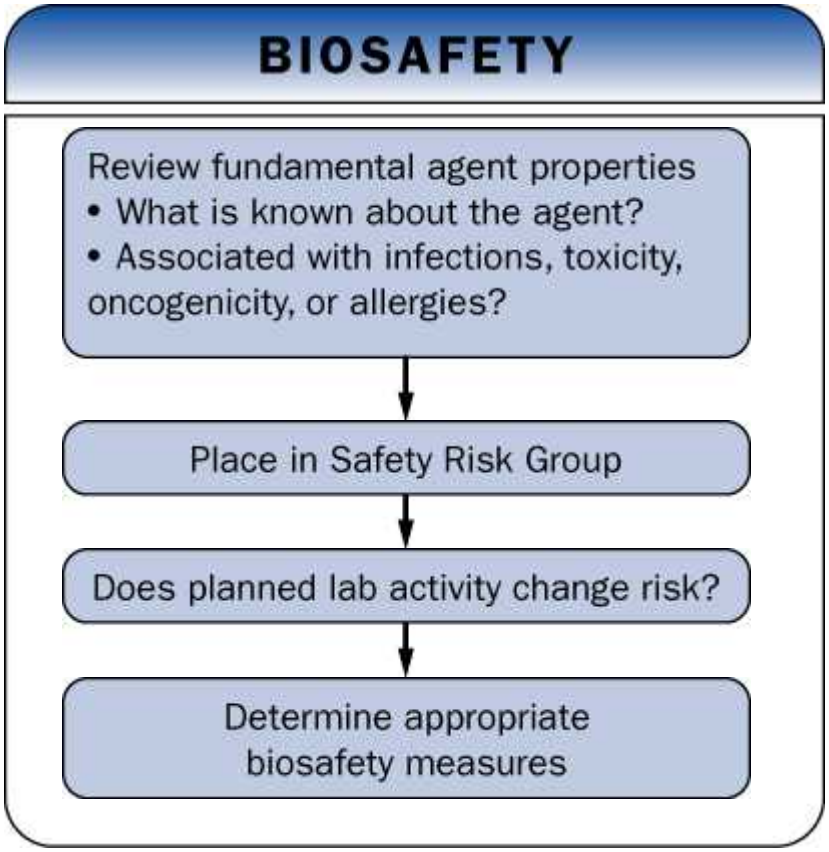
- Aerosol Exposure
  - Centrifugation
  - Pipetting
  - Homogenizing (vortexing, grinding, or blending)
  - Sonication, heating or boiling
  - Loop flame-sterilization
  - Flow cytometry
- Containers with clinical specimens
- Animal studies
  - Non-human primates
  - Litter and animal waste
- Skin puncture
- Tubercle bacilli have been reported to survive heat-fixed smears
- Frozen material when cut can release ice particles which are contaminated, even if formalin-fixed



Recommended precautions/practices

- Risk Assessment
- Containment
- PPE
- Surface Decontamination
- Waste Decontamination
- Inactivation
- Incident response

Risk Assessment



Containment

- Health Canada and the CDC recommend:
  - Biosafety level 2 practices, containment equipment and facilities for primary culture of sputum and smear preparation
  - Biosafety level 3 practices, containment equipment and facilities for the propagation and manipulation of cultures of *M. tuberculosis* or *M. bovis* and for animal studies utilizing non-human primates.
- Work should be conducted within a BSC when possible
- Work with MDR or XDR
  - Based upon risk assessment, any non-diagnostic biosafety level 3 containment is recommended p1
  - Class III BSC should be considered for:
    - Aerosol studies
    - Studies with large cultures

Slide 10

p1 is this stating that BSL3 containment is required for non-diagnostic work?
paustin, 5/16/2007

PPE

- **Gross contamination protection**
  - **Laboratory coat and gloves when manipulating TB specimens**
  - **Gloves and gown with tight wrists and ties in back when manipulating TB cultures**

  - **If working with chemicals, the selection of gloves used should be based upon the chemical***
    - **E.g. If working with Tetrahydrofuran (THF) only Teflon gloves provide protection**

- **Eye and mucosal membrane protection**
  - **Goggles or facemask should be worn manipulating TB specimens or cultures**
    - **A full facemask protects against unintentional touching of the mouth, nose and eyes with a contaminated hand**

*See glove/chemical chart

PPE (Respiratory Protection)

- Respiratory protection for:
  - Work outside the BSC
  - Any work with MDR/XDR
  - High level of aerosolization risk

  - Surgical masks do not provide any protection from TB
    - Infectious droplet nuclei < 5 um in diameter
  - Particulate mask respirator
    - N, R and P models
    - N100 is effective for working with TB
    - Medical assessment and fit testing are required with particulate masks
  - Powered Air purifying Respirator (PAPR)

PPE (Blood borne pathogen protection)

- Blood borne pathogen protection
  - Blood borne pathogen protection when sharps (including potential sharps like glassware) are in use
  - Gloves
    - Multiple layers of gloves can reduce the amount of infected material on a sharp instrument when it punctures the skin
    - Heavy weight utility gloves should be worn for equipment cleaning and spill response
  - Sharps handling
    - Utilize safe sharp devices
    - Keep hands away from needles
    - Use mechanical methods for needle removal
    - Never bend, recap or manipulate sharps by hand.
    - Dispose of entire unit into sharps container
    - Collect reusable sharps in labeled, leak-proof container

Surface Decontamination

- M. tuberculosis has a high lipid content of the cell wall which creates a greater resistance to classical disinfectants
- MDR and XDR strains do not show any difference from the classical strains

- Ineffective Disinfectants:
  - Quaternary ammoniums only inhibit
  - Resistant to acids, alkali and mercurial compounds
- Effective Disinfectants:
  - 5% Phenol or 5% formaldehyde - 10 minute contact
  - 2% Glutaraldehyde - 30 minutes contact time
  - 5% Sodium hypochlorite - 1 minute contact time
  - 70% Ethyl and isopropyl alcohol
  - Iodine and ionophores are also effective when used with ethyl alcohol

Waste Decontamination

- Articles should be autoclaved at a minimum temperature of 121°C & 1 MPa (15 psi) for a minimum period of 15 minutes
  - After autoclaving waste material may be disposed of as rubbish
  - Re-usable articles may be washed and reused
- Animals larger than mice cannot be fully decontaminated via autoclaving*
  - Mice require 1.5 hours in autoclave to be fully decontaminated
- Autoclaving can be used to decontaminate the surface of an animal storage container
  - Animal carcasses should be incinerated or placed into a chemical digester

*RTI International 1989

Inactivation

- Traditional Chemical Inactivation
  - 2% paraformaldehyde and 2% glutaraldehyde
  - 5% formalin
- Chemical inactivation study:
  - 90% ethanol for 2 hrs at room temperature then incubated at 96*C in 20% Chelex for one hour showed 100% inactivation (Djelouagji et al)
- Heat Inactivation studies:
  - Heat inactivation of TB at 80°C was shown to not be effective
    - 77% of tested cultures were shown to still be active
  - Heat inactivation in a 100°C water bath or dry heat oven at 95°C for 20 min showed inactivation but also degraded the DNA. (Seagar et al)
- All inactivation's must be validated regardless of method before handling at a lower biosafety level

Incident response

- **Spill Response**
  - Allow aerosols to settle
  - Wearing protective clothing, gently cover spill with paper towels and apply 5% phenol, starting at perimeter and working towards the centre
  - Allow sufficient contact time before clean up
  - Decontaminate before disposal

- **Post Exposure**
  - Incident should documented in writing
  - The effected person(s) should be counseled immediately after exposure and referred a physician or health department to begin follow up and appropriate therapy
    - Baseline testing should be performed as soon as possible post-incident
  - Any person should be clinically evaluated for active tuberculosis; if active tuberculosis is diagnosed, appropriate therapy should be initiated
  - Others within the laboratory should also be tested if the exposed individual is positive

Medical surveillance

- Persons working with TB should have a tuberculin skin test, unless a previously positive reaction can be documented or after completion of appropriate preventative therapy or adequate therapy can be documented
- Persons with a history of Bacillus of Calmette and Guerin (BCG) vaccination should also have the tuberculin skin test
- Persons who exhibits a first time positive reaction to the skin test must be cleared by a physician
  - Investigation of exposure route
  - Additional testing required as TB is endemic
- Persons with a history of a positive skin test (PPD) or adequate treatment of latent infection or active diseases should be from further testing unless signs and symptoms of TB disease develop
- Periodic retesting of PPD-negative persons be conducted to identify persons whose skin tests convert to a positive status
  - The frequency of retesting is risk-dependent

Medical surveillance (con't)

- **Skin Testing**
  - **Targeted tuberculin skin testing (TST) uses a purified protein derivative which is also within the BCG vaccine**
  - **People who have had the BCG vaccine should be retested 6 weeks after the initial test to look for reaction to the vaccine**
- **New whole blood tests are showing more accurate results then the TST in areas where BCG vaccine is common.**
  - **QFT-RD1 is one of these tests**

References

- Health Canada Material Safety Data Sheet
- Biosafety Recommendations for the Contained Use of *Mycobacterium tuberculosis* complex isolates in industrialized countries, Royal Library of Belgium
- Interim Laboratory Biosafety Guidance for Extensively Drug-Resistant (XDR) *Mycobacterium tuberculosis* strains, Centers for Disease Control (USA)
- Goals for working safely with *Mycobacterium tuberculosis* in Clinical, Public Health and Research Laboratories, Department of Health and Human Services (USA)